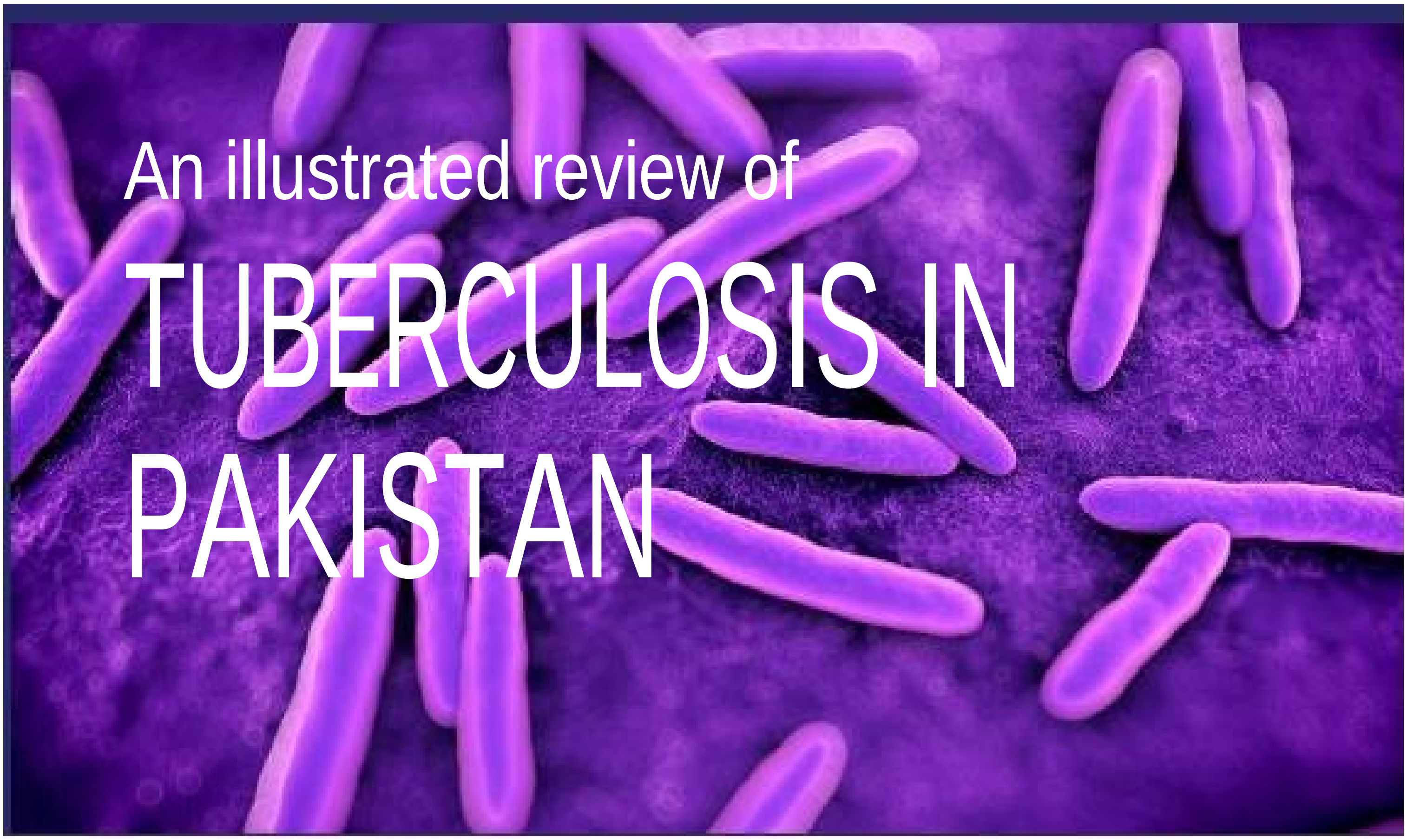

An illustrated review of
TUBERCULOSIS IN PAKISTAN

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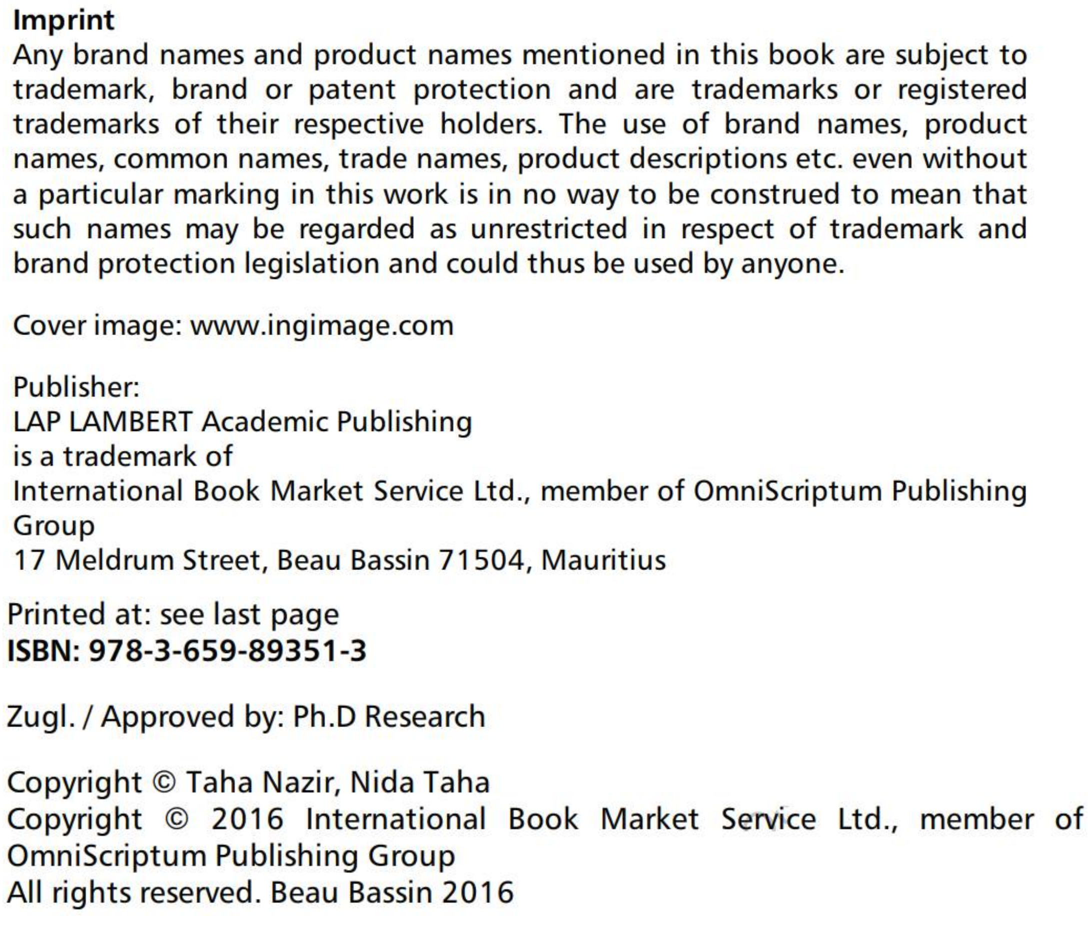

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PREFACE

The dangerous resistant bacterial strains or superbugs are potential threats. That needs special attention to address the problem appropriately. The multi-drug resistant and extensive drug resistant *M. Tuberculosis* are emerging rapidly because of non-compliance and unsupervised indiscriminate use of antibiotics. The cases of Tuberculosis cases have been reported in highly susceptible population groups including the local population of Pakistan.

Tuberculosis is one of the major public health problems in Pakistan. That ranks 5th amongst high burdened countries of the world. There are 61% of the Tuberculosis patients in the WHO Eastern Mediterranean Region. Approximately 420 000 new Tuberculosis cases emerge every year and half of these are sputum smear positive.
Pakistan is also estimated to have the 4th highest prevalence of multidrug resistance globally.
Hence, an effective strategy is desired to control Tuberculosis here in Pakistan. We have tried to present comprehensive clinical and microbiological information related to Mycobacterium Tuberculosis. That helps to develop the required skills to achieve the Millennium Development Goals of World Health Organization in Pakistan.

The valuable feedback and constructive suggestions will warmly be welcomed.

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TERMINOLOGIES

1 **Tubercular Patient**: An individual with identified isolated of drug susceptible/ resistant *Mycobacterium Tuberculosis* referred as Tuberculosis patient. He may have prior treatment history or documentation of previous Tuberculosis disease.

2 **Primary Tuberculosis**: 1st exposure/ attack of MTuberculosis, characterized by formation of primary complex, parenchymal pulmonary lesion and a small subapical focus.

3 **Secondary Tuberculosis**: It is the active infection in previously sensitized individual. It is Mostly due to reactivation of dormant bacilli from primary lesion. Occasionally the reason observed from an exogenous source.

4 **Extrapulmonary Tuberculosis**: Tubercular Pathogenicity/ infection other than lungs referred as Extrapulmonary Tuberculosis.

5 **Drug Resistance**: It is defined Tuberculosis caused by *M. Tuberculosis* bacilli resistant to one or more anti-Tuberculosis agent. Drug resistance is further classified into "primary", "initial" or "acquired" according to history of previous Tuberculosis treatment.

6 **Initial Drug Resistance:** Includes primary as well as unknown and undiagnosed acquired drug resistance referred as Initial Drug Resistance. (9/I)

7 **Primary Drug Resistance**: Resistance in cultures from patients with no history of previous Tuberculosis treatment. 56/ D6 or Resistance in patient who has never taken drugs in the past and gets infected with strain originating from other patient who had acquired resistance.

8 **Acquired Resistance**: Resistance acquired after intake of drugs by patients referred as acquired drug resistance (9/I) or Resistance in cultures from patients with one or more previous Tuberculosis treatment episodes (totaling more than one month).

9 **Natural Drug Resistance**: Neither the patient with naturally resistant bacilli nor his source of infection has had chemotherapy in the past. or selective propagation of spontaneously resistant mutants in a wild population. Tubercle bacilli have spontaneous predictable rates of chromosomally borne mutation and propagation of mutants into population leads to resistance. Naturally occurring mutants occur independent of exposure to chemotherapeutic agents and resistance is usually to a single drug.

10 **Multi-Drug Resistant (MDR) Tuberculosis** ***MDR-Tuberculosis***: *Mycobacterium Tuberculosis* resistant to Isoniazid and Rifampicm with/without resistance to other drugs. (9/I)

11 **Primary Chemotherapy Failure**: Tuberculosis in newly diagnosed patients who remain smear positive after six months standard chemotherapy.

12 **Relapse Cases**: Recurrence of Tuberculosis within one year in a patient who had been declared cured in the past by physician.

13 **Chronic Cases**: Tuberculosis in a patient who remains smear positive after completing retreatment regimen under supervision or the failure of a fully supervised retreatment regimen. A chronic case has received at least two courses of chemotherapy, and

sometimes more than two courses (complete or incomplete). Chronic cases are often, but not always, excreters of MDR bacilli. Likewise, patients with retreatment failure are more likely to be harboring multidrug resistant organisms.

14 **Drug Defaulters**: Who consumes less than 80% of the prescribed drugs either in duration or doses or both.

15 **Treatment Failure:** A Tuberculosis patient who remains or becomes again smear-positive at five months or later during treatment. is still excreting bacilli at the end of treatment (at five to six months for new cases or seven to eight months for retreatment cases).

16 **Molecular Epidemiology**: The genetic level molecular study of Tuberculosis to evaluate the ouTuberculosisreak is referred as molecular epidemiology.

17 **Tuberculosis diagnosis date**: It can be expressed as the collection date of the first specimen from which an *Mycobacterium Tuberculosis* isolate was cultured.

18 **Homelessness:** It is defined as being in a public or private shelter or having no address at the time of the Tuberculosis diagnosis.

19 **RFLP:** Restriction Fragment Length Polymorphism.

20 **SSCP PCR**: Single Strand Conformation Polymorphism PCR

21 **Luciferase reporter mycobacteriophage test**: MTuberculosis infecting virus mycobacteriophage with a cloned gene for production of Luciferase reporter enzyme used to elucidate the sensitivity. The LRM when mixed with culture of bacterial cell result in production of light.

22 **EESBM**: Egg enriched sheep blood media used for rapid growth of MTuberculosis.

23 **MGIT Method**: Microbial growth indicator tube method used to identify the susceptible/ resistant MTuberculosis.

24 **IID Method**: International Immune diagnosed method used to identify the MTuberculosis or its antigens for diagnosis purpose.

25 **Radiometric BACTEC Method**: Becton Dickinson radiometric method of detection of mycobacterial growth by sophisticated gamma camera.

26 **Chemoprophylaxis**: Prophylactic administration of anti-Tuberculosis agents to reduce the disease risk referred as chemoprophylaxis.

Chapter 1.

INTRODUCTION OF TUBERCULOSIS IN PAKISTAN

There are around 161 million people live in an area of 803,490 square kilometers in Pakistan. Every year Tuberculosis causes approximately 70,000 deaths, around 270,000 people per year fall sick of the disease. According to WHO the country is among the 27 most affected states, meanwhile this holds true for (Multi Drug Resistance Tuberculosis) MDR-Tuberculosis. (DAHW, 2016) Annually approximately 15,000 patients contract this severe form of Tuberculosis; the standard antibiotics do not work anymore. The treatment of MDR-Tuberculosis is lasting longer and is more expensive than the treatment of the common Tuberculosis. Besides, it has severe side effects. (Pedro et al., 2015)

The hospital cares for patients who are not covered by the network of the governmental Tuberculosis program. The hospital admits, for example, malnourished, severely sick people and very poor patients, who cannot be cared for at home. Patients suffering from extra pulmonary Tuberculosis, for example vertebral Tuberculosis will be helped here. The treatment of MDR-Tuberculosis is gaining more and more importance. Since, up to now, there has only been a government program for MDR-Tuberculosis in Lahore and Karachi, even the government hospitals of Northern Pakistan are referring these patients to Dr. Schmotzer, unfortunately often much too late. Patients suffering from this highly infectious Tuberculosis are treated in two small isolation wards with four to five beds each, in order to avoid infection of other patients. By now there is a waiting list for admission to this ward. Prevention: The team in Rawalpindi especially emphasizes the education of the public and the training of health staff. This is the reason why up to now no patients and co-workers have been infected by Tuberculosis while staying at the hospital. (DAHW, 2016)

- Total population 184 350 (x000s)
- Total health expenditure (% of general government expenditure) 4.7
- Maternal mortality ratio (per 100 000 live births) 170
- Primary health care centers and units (per 10 000 population) 2.0
- Total life expectancy at birth (years) 65.0
- Source: Country statistical profiles (2014)

Tuberculosis (Tuberculosis) is one of the major public health problems in Pakistan. Pakistan ranks fifth amongst Tuberculosis high-burden countries worldwide. It accounts for 61% of the Tuberculosis burden in the WHO Eastern Mediterranean Region. Approximately 420 000 new Tuberculosis cases emerge every year and half of these are sputum smear positive. Pakistan is also estimated to have the fourth highest prevalence of multidrug-resistant Tuberculosis (MDR-Tuberculosis) globally. (Singh et al., 2015)

Steady progress has been made from 2001 onwards to improve the Tuberculosis case detection and treatment success rate. This has been achieved by quality assurance of smear microscopy, drug management community mobilization and involving tertiary care hospitals, nongovernmental organizations, inter-sectoral organizations and the private sector in service delivery.

The number of Tuberculosis cases diagnosed increased from 20,707 in 2001 to 267,912 in 2010. Since its revival in 2001, the national Tuberculosis control program has successfully treated more than 1.5 million Tuberculosis cases, free of cost.

The current case detection rate of new smear positive Tuberculosis cases is 63% based on new estimates of incidence, and the treatment success rate is 91%. A total of 5800 diagnostic and treatment centers providing free Tuberculosis testing and treatment services have been established in the public sector.

MDR-Tuberculosis management has started at three pilot sites. Resources have been secured for comprehensive management of 15 000 MDR-Tuberculosis patients (diagnosis, treatment and social support).

Incidence of Tuberculosis in Pakistan (per 100,000 people)
Incidence of Tuberculosis (per 100,000 people) in Pakistan was last measured at 270 in 2014, according to the World Bank. Incidence of Tuberculosis is the estimated number of new pulmonary, smear positive and extra-pulmonary Tuberculosis cases. This page has the latest recorded value, an historical data chart and related indicators for Incidence of Tuberculosis (per 100,000 people) in Pakistan. (Taha et al., 2009)

Epidemiology of Tuberculosis in Pakistan

There is little reliable epidemiological data available for Pakistan, although Tuberculosis is considered to be a major cause of ill health. (Khan, 2001) The annual incidence rate of infectious Tuberculosis cases is estimated to be between 85-100/100,000 persons. Annually around 120,000 new Tuberculosis cases are being added to the existing number of infectious individuals. Some areas in the country have much higher figures, such as Northern Pakistan where prevalence figure of 554/100,000 cases was observed. (Alvi et al., 1998)

As in other developing countries, young age groups are affected the most. Male patients outnumber females in most age groups, except in the adolescents. Based on Burden of Disease estimates, Tuberculosis represents 5% of the total DALYs (Disability Adjusted Life Years). Which indicates that the burden of Tuberculosis in Pakistan. is substantially higher than the world average of 3%.

Gender comparison

Data showed that there were 70.9% males and 29.1% females of Tuberculosis diagnosed patients of age 17- 67 years in Pakistan. Gender comparison depicts greater percentage of male than females. Uplekar et al., (2001), reported a 70% excess of male over female Tuberculosis cases globally each year, the reasons for this difference are unclear. Generally, women in poor countries confront more barriers than men in accessing health care services. Yet, research is lacking to explain the impact of gender inequalities in access to care on reported sex ratios for Tuberculosis. A review of the limited available literature and field visits to Tuberculosis programs offered insights and suggested a framework to study gender differentials in Tuberculosis. That considers the role of gender at various steps in effective Tuberculosis care. Moreover, Haq et al., (2002), reported 68% males and 32% females Tuberculosis patients. WHO, (2004), reported approximately 67% of males Tuberculosis patients. Moreover, Bitar et el., (2001), reported 70% males with a male female ratio of 2:8. For several other demographic and clinical characteristics information was missing for an important proportion of cases. The possible reasons may because of the facts reported by Jiménez, (2006). Who described the most low income countries have twice as many cases of Tuberculosis among men than among women. A difference commonly attributed to biological and epidemiological characteristics as well as socioeconomic and cultural barriers in access to health care.

The pulmonary and extra-pulmonary Tuberculosis

There were 84.30% pulmonary and 15.69% extra-pulmonary specimens from Tuberculosis patients.

The pulmonary Tuberculosis patients were diagnosed by collecting the biological samples of sputum, bronchial washing & puss, while the extra pulmonary Tuberculosis were idndentified by clinical isolates of puss, blood and biopsy fluids. Bitar et el., (2001), reported the majority 89% (n=607;) cases with pulmonary Tuberculosis, 6% (n-39) Extra pulmonary Tuberculosis (EPTuberculosis); and 5% (n-37) cases for whom site of disease was unknown.

Tuberculosis control in Pakistan

The first survey was carried out in Pakistan during1962. The collaborative efforts of Ministry of Health, Government of Pakistan were become fruitfully conclusive. WHO and UNICEF for a twenty year Tuberculosis control program. That focused on establishing specialized Tuberculosis centers and special Tuberculosis wards at the DHQ Hospitals. In 1985 UNICEF withdrew its financial support. WHO declared Tuberculosis a global emergency in 1993 and the Govt. of Pakistan endorsed the DOTS strategy. In 1994, Ministry of Health, in collaboration with WHO revised the Tuberculosis control policy. National policy and technical guidelines were drafted: however, to date there is no draft yet for operational guidelines. In 1995 the Ministry of Health decided on the location of 5 DOTS pilot sites, but only 1 site became operational. A highly centralized and vertical live-year development plan was prepared by the Federal National Tuberculosis Control Program (NTP). Since the Provinces expressed certain reservations with regard to the plan, it was not approved. In 1996 the Directorate for Tuberculosis Control of Pakistan was abolished and the MS of the Tuberculosis Centre in Rawalpindi made responsible for National Tuberculosis Control program, but without any additional support. In 1998 Pakistan was declared I of the 16 countries without an appropriate NTP. Recently it was decided that each province would be responsible to plan and manage its own NTP under Federal NTP guidelines. Funding for the plans will be provided through Second Social Action Program Project II (SAPP II).

National Tuberculosis Control Program Pakistan

Given the magnitude of the Tuberculosis problem in Pakistan as well as the size of the country, a vertical Tuberculosis control program is financially prohibitive and difficult to sustain. Integration of the Tuberculosis control program in PHC has recently been opted for, as a solution to its technical and managerial deficiencies. Hereto, a network of laboratories needs to be created as well as a system for ensuring quality of sputum smear microscopy put in place.

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Chapter 2.

PARTNERSHIP OF WORLD HEALTH ORGANIZATION TO CONTROL Tuberculosis IN PAKISTAN

Tuberculosis constitutes a major public health challenge. Due to the emergence of lily, increased migration and the deterioration of the health services in many countries, the incidence has risen so drastically in recent years, that Tuberculosis was declared a global emergency by WHO in 1993. (WHO, 1998) Without increased investment in intervention strategies, the global Tuberculosis situation is expected to worsen in the near future. (MeKinney et al., 1998)

WHO support for Tuberculosis control in Pakistan

WHO has been supporting the national Tuberculosis program since 2001. The support is focused on capacity-building at all levels: national, provincial and district. In particular, support has been in the following areas:

1. Program evaluation
2. Technical assistance for the management of MDR-Tuberculosis
3. Mobilization of resources through the Joint One United Nations Initiative
4. Support for research and development. (WHO, 2015)

The WHO Representative’s Office in Pakistan supports the Government and health authorities in strengthening health services, addressing public health issues and supporting and promoting research for health. Physicians, public health specialists, scientists, social scientists and epidemiologists provide appropriate technical support and collaboration upon the request or acceptance of national authorities.

As the lead health agency, WHO works with many partners to support countries in reaching their national health development goals and to ensure that its efforts are coordinated. Across the Region, these include United Nations agencies, humanitarian and development partners, donors, nongovernmental organizations, WHO collaborating centers and the private sector. WHO works with the Planning Commission, Pakistan, to agree on the collaborative program of work for WHO and the Government of Pakistan at the federal level, and to promote the inclusion of health in all policies and national development plans. WHO also works with the Inter Provincial Coordination Ministry, to ensure coordination on health issues with provincial health authorities, and with the Economic Affairs Division for donor coordination.

However, since the 18th Amendment to the Constitution of Pakistan, provincial health departments have become the main actors for both planning and implementation of health program and service delivery. Since the establishment of its country office in Pakistan in 1960, WHO has provided a wide range of technical support to the Government of Pakistan. This has been mainly in the health sector across a wide spectrum of health-related activities ranging from policy, strategic planning, health system and community development, health promotion and communicable disease control. Working within the overall framework of the WHO mandate and in line with governmental priorities and responsibilities, Country Cooperation Strategies have been developed for six-year periods, reflecting the medium term strategic vision. In addition, joint collaborative program have been developed for each biennium to outline specific areas of cooperation between the two partners. The major areas of WHO collaboration with the Government of Pakistan are technical support for:

1. health sector policy and reforms
2. health system strengthening, including capacity-building of the human workforce for health
3. Expanded Programe on Immunization and the Polio Eradication Initiative
4. Disease Early Warning System

5. control of Tuberculosis, HIV/AIDS and malaria
6. family planning and primary health care
7. emergency preparedness and humanitarian assistance
8. provision of essential medicines
9. assisting in providing safe water and other environmental health issues
10. community-based initiatives
11. noncommunicable disease prevention and control, including the Tobacco Free Initiative, promotion of healthy lifestyles, prevention of avoidable blindness and mental health.

Through the United Nations Delivering as One initiative in Pakistan, WHO is also converging the efforts of over 12 United Nations agencies, offices and funds in the health and population sectors to help achieve the Millennium Development Goals.

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Chapter 3.

SUSCEPTIBILITY AND RESISTANCE PATTERN OF MYCOBACTERIUM TUBERCULOSIS AGAINST 1ST LINE ANTI-TUBERCULOSIS DRUGS

There were 70.9% males and 29.1% females' patients of Tuberculosis of age 17- 67 years in Pakistan. Gender comparison depicts greater percentage of male than females. Uplekar et al., (2001) reported a 70% excess of male over female Tuberculosis cases globally each year, the reasons for this difference are unclear. Generally, women in poor countries confront more barriers than men in accessing health care services. Yet, research is lacking to explain the impact of gender inequalities in access to care on reported sex ratios for Tuberculosis. A review of the limited available literature and field visits to Tuberculosis programs offered insights and suggested a framework to study gender differentials in Tuberculosis. That considers the role of gender at various steps in effective Tuberculosis care. Moreover, Haq et al., (2002), reported 68% males and 32% females Tuberculosis patients. WHO/ IUALTD Report No. 2, (2000), reported approximately 67% of males Tuberculosis patients. Moreover, Bitar et el., (2001), reported 70% males with a male female ratio of 2:8. For several other demographic and clinical characteristics information was missing for an important proportion of cases. The possible reasons may because of the facts reported by Jiménez, (2006). Who described the most low income countries have twice as many cases of Tuberculosis among men than among women. A difference commonly attributed to biological and epidemiological characteristics as well as socioeconomic and cultural barriers in access to health care.

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The pulmonary Tuberculosis patients were diagnosed by collecting the biological samples of sputum, bronchial washing & puss, while the extra pulmonary Tuberculosis were identified by clinical isolates of puss, blood and biopsy fluids. Bitar et el., (2001), reported the majority 89% (n=607;) cases with pulmonary Tuberculosis, 6% (n-39) Extra pulmonary Tuberculosis and 5% (n-37) cases for whom site of disease was unknown.

Sensitivity/ resistance of M. Tuberculosis against 1stline anti-Tuberculosis drugs

The standard minimum inhibitory concentration of anti-Tuberculosis drugs rifampicin, isoniazid, ethambutol and pyrazinamide were 40 µg/ml, 0.2µg/ml, 2µg/ml and 100µg/ml respectively. These concentrations can be incorporated into the media and consider as border line for declaration of sensitive or resistant mycobacterium. The Clinical complications such as emphysema and extensive cavitation permit a large population to develop in a compartment into which drugs may not penetrate. This large bacterial pool increases the population for mutation, and with poor penetration there is an increased likelihood of resistance emerging. A similar situation may develop in patients with extensive disease or poor immunity (Stephen, 2002). Tuberculosis control still faces major challenges in Pakistan. One of the maxims of Tuberculosis control has been inadequate therapy which is worse than no therapy at all. It is established that incomplete corner of Tuberculosis treatment would result in high rates of failure and rapid creation of drug resistance (Rizwan et al., 2003). Drug resistant Tuberculosis is increasing day by day and is a significant threat to Tuberculosis control because there are few drugs effective against Mycobacterium Tuberculosis (Bengisun et al., 2000). The association between length of treatment and drug resistance may reflect longer treatment as a result of treatment failure in patients with drug resistance; it may also reflect irregular prior treatment for Tuberculosis, leading to drug resistance (Espinal et al., 2001).

Sensitivity of *Mycobacterium Tuberculosis* against Rifampicin

During a study in Pakistan the clinical samples of *Mycobacterium Tuberculosis* were collected from Tuberculosis diagnosed patients. 21.5% strains were found resistant and 78.5% sensitive against rifampicin. (Taha et al., 2010; WHO/ IUALTD, 2000), reported the rifampicin resistance in India 25%.and in Estonia 39%. Drug resistance complicates efforts to control Tuberculosis, besides MDR-Tuberculosis the increasing resistance of rifampicin is alarming (Haq et al., 2002). Moreover, Herendra & Shah, (1998), conceded 20-30% rifampicin resistance. Rizwan et al., (2003), reported a total of 678 confirmed isolates of Mycobacterium Tuberculosis subjected to anti Tuberculosis drug resistance. Five anti-Tuberculosis drugs were studied for drug sensitivity pattern. They include rifampicin, isoniazid, pyrazinamide, streptomycin and ethambutol. 53% of isolates were resistant to one or more anti Tuberculosis drugs, resistance to rifampicin came out to be 28%, while resistance to MDR was 16%. Primary and acquired resistance was 16% and 44% for rifampicin. Statistically significant difference was seen between primary and acquired resistance. It is seen that Tuberculosis control can be achieved with more focus on early case detection and prompt treatment. The global expansion of DOTS is now a day's standard strategy for efficient control of Tuberculosis. While, Miah et al., (2000), reported an overall resistance came out to be 30% in Bangladesh. That showed11% resistance to rifampicin with 5% MDR strains. Similar findings were reported by Sumathi & Srivastava, (2004), who carried out a study to establish a rapid and accurate method of susceptibility testing for Mycobacterium Tuberculosis using three methods. They designed the proportion method by agar dilution on Middlebrook 7H11 agar, proportion method using the conventional Lowenstein-Jensen (L-J) medium and E test strip method. 67 (89.3%) isolates were sensitive to rifampicin by the three methods respectively and 6 (8.0%) isolates seen resistant to rifampicin by all the three methods. Of the 75 samples, 52 (69.3%) were sensitive to all the 5 drugs by the L-J medium proportion method. 49 (65.3%) were sensitive to all the five drugs by all the three methods. One (1.3%) isolate alone was resistant to all the five drugs by the three methods. The possible reason of difference in results may lie in the facts of the priority of research objectives; methodology adopted and proposed protocol to conduct the project. There also demographic and socio-economic factors influence the research outcomes.

Table: Level of resistance (in % age) of rifampicin resistant *Mycobacterium Tuberculosis*

Rifampicine level in LJ Media	Rifampicin ug/ ml	Percent resistance	Valid Percent	Cumulative Percent
1	40	100		
2	80	100		
3	120	40.541	40.541	40.541
4	160	35.135	35.135	75.676
5	200	18.919	18.919	94.595
5+	200+	5.405	5.405	100
	Total	100	100	

Sensitivity of *Mycobacterium Tuberculosis* against Isoniazid

0.2µg/ml is an optimum isoniazid concentration that can be incorporated into the media. This concentration is considered as the border line for declaration of sensitive or resistant Mycobacterium. A study reported 14.5% strains resistant and 85.5% sensitive to isoniazid of total clinical isolates of Mycobacterium

Tuberculosis in Pakistan. (Taha et al., 2011) While, WHO/ IUALTD, (2000), reported the 19.4% isoniazid resistance in Russian Federation, 11.3 % resistance in china and 15.4 % resistance in India. CCDR (Canada Communicable Disease Report), (2004), reported 9.3% resistance against isoniazid.

Table: Level of resistance (in % age) of isoniazid resistant *mycobacterial Tuberculosis*

Isoniazid Levels in LJ Media	Isoniazid ug/ ml	Percent resistance	Valid Percent	Cumulative Percent
1	0.2	100		
2	0.4	100		
3	0.6	36	36	36
4	0.8	28	28	64
5	1	24	24	88
5+	1+	12	12	100
	Total	100	100	

The possible reason of difference of results may lie in the facts of the differences in living standards, health facilities, over all hygienic condition and socio-economic structure. Moreover, Herendra & Shah, (1998), reported 20-30% isoniazid resistance. Whereas, Stephen, (2002), reported some physiological conditions may induce a hypermutable state, making multiple resistances more likely. The assumption that resistant organisms are less fit than wild-type strains may not be correct, as the initial fitness deficits may be attenuated by adaptation by multiple passages. Instances of isoniazid resistance, where attenuated virulence is common, may occur because the molecular mechanism of resistance directly affects a system required by the organism for intracellular survival. The important lesson these clinical and molecular studies teach us is that resistant organisms over time will be fully virulent and that if we are to prevent an epidemic of multiple drug- resistant Tuberculosis we must take steps to ensure that all patients are diagnosed and effectively treated so that resistant strains are not created and transmitted in the community. Miah et al., (2000), reported 15.8% resistance against isoniazid and 5% to MDR in Bangladesh. Whereas, Sumathi & Srivastava, (2004), reported a rapid and accurate method of susceptibility testing for Mycobacterium Tuberculosis using three methods proportion method by agar dilution on Middlebrook 7H11 agar, proportion method using the conventional Lowenstein- Jensen (L-J) medium and E test strip method. A total of 56 (74.7%) isolates were sensitive and 11 (14.7%) were resistant toisoniazid by the three methods. 11 24(14.7%) isolates were seen resistant to isoniazid by all the three methods. 49 (65.3%) were sensitive to all the five drugs by all the three methods. One (1.3%) isolate alone was resistant to all the five drugs by the three methods. Rizwan et al., (2003), reported a total of 678 confirmed isolates of Mycobacterium Tuberculosis subjected to anti Tuberculosis drug resistance. Five anti Tuberculosis drugs were studied for drug sensitivity pattern. They include rifampicin, isoniazid, pyrazinamide, streptomycin and ethambutol. 53% of isolates were resistant to one or more anti Tuberculosis drugs. The resistance to isoniazid came out 26%, while resistance to5MDR was 16%. Primary and acquired resistance was 20% and 33% for isoniazid.5Statistically significant difference was seen between primary and acquired resistance.

Sensitivity of Mycobacterium Tuberculosis against Ethambutol

The critical concentration of Ethambutol that can be maintained into the media to consider as border line for declaration of resistant is 2µg/ml. Taha et al (2010) reported 5.8% resistant and 94.2% sensitive strains to ethambutol of total clinical isolates of Mycobacterium Tuberculosis in Pakistan.

Table: Level of resistance (in % age) of ethambutol resistant *mycobacterium Tuberculosis*

Ethambutol Levels in LJ Media	Ethambutol ug/ ml	Percent resistance	Valid Percent	Cumulative Percent
1	2	100		
2	4	100		
3	6	50	50	50
4	8	30	30	80
5	10	20	20	100
	Total	100	100	

WHO/ IUALTD, 2000, reported ethambutol resistance as 5.8% in Iran and 4.1% resistance in china. Bitar et el 2001, reported 4.4% resistant against ethambutol. While, Khan et el (2001), who reported drug resistance in Saudi Arabia has markedly increased during the last 5 years, except for pyrazinamide and ethambutol showing resistance of 8% & 7%. Whereas, Miah et al., (2000), reported 2.7% resistance against ethambutol in Bangladesh. Moreover, Sumathi & Srivastava, (2004), reported a study to establish a rapid and accurate method of susceptibility testing for Mycobacterium Tuberculosis. 70 (93.3%) isolates were sensitive and 2 (2.7%) isolates were resistant to ethambutol by all the three methods. Rizwan et al., (2003), reported an indigenous research of 53% resistant against one or more anti Tuberculosis drugs and resistance to ethambutol came out to be 15%. Primary and acquired resistance was 512% and 19% for ethambutol. Statistically significant difference was seen between primary and acquired resistance. Whereas, Bertram, (2004), has confirmed the resistance of Mycobacterium Tuberculosis against ethambutol.

Sensitivity of Mycobacterium Tuberculosis against Pyrazinamide:

The susceptibility pattern of Tuberculosis patients for pyrazinamide was found as 27.3% resistant and 72.7% sensitive of total clinical isolates of Mycobacterium Tuberculosis. (Taha et al., 2008)

Table: Level of resistance (in % age) of pyrazinamide resistant *mycobacterium Tuberculosis*

Drug Levels in LJ Media	Pyrazinamid ug/ ml	% Resistance	Valid Percent	Cumulative Percent
1	100	100		
2	200	100		
3	300	27.660	27.660	27.660
4	400	25.532	25.532	53.191
5	500	31.915	31.915	85.106
5+	500+	14.894	14.894	100
	Total	100	100	

Bitar et al., (2001), reported 25% resistant to pyrazinamide (one-third of cases with missing information). Joal., (2001), reported the resistance prevalence and molecular level comprehension of resistance against pyrazinamide. Whereas, Rizwan et al., 2003, reported a total 678 confirmed isolates of Mycobacterium

Tuberculosis subjected to anti Tuberculosis drug resistance. Five anti Tuberculosis drugs - rifampicin, isoniazid, pyrazinamide, streptomycin and ethambutol were studied for drug sensitivity pattern. The resistance to pyrazinamide came out to be 29% and 53% of isolates were resistant to one or more anti Tuberculosis drugs. Primary and acquired resistance was 19% and 39% for pyrazinamide.Statistically significant difference was seen between primary and acquired resistance.

Mono-resistance profile against 1stline anti-Tuberculosis drugs

Mono-drug resistance of Mycobacterium Tuberculosis was studied against 1stline anti-Tuberculosis drugs. The overall comparison has shown 25.71% strains resistant to rifampicin, 8.57% strains resistant to isoniazid, 2.85% resistant to ethambutol and 62.85% resistant to pyrazinamide.(Nazir et al., 2015) Whereas, 20.34% strains were found mono drug resistant. Helen, (2002), reported similar findings in 2002. 268 (69.6%) of total 385 cases of Tuberculosis were reported by the Mycobacteriology Reference Laboratories as culture positive. Antimicrobial susceptibility testing results were available for all 268 isolates, which comprised 264 Mycobacterium Tuberculosis and four M. bovis isolates. The proportion of isolates resistant to isoniazid is 9.7%, rifampicin 1.5%, ethambutol 1.9% and pyrazinamide 4.1%. Over the last eight years, there has been a trend of increasing streptomycin resistance (p=0.0078). Isoniazid and pyrazinamide resistance has fluctuated, while rifampicin and ethambutol resistance has been relatively stable and remained ≤2%. There significant differences observed in resistance profile of rifampicin and pyrazinamide. The possible reasons may lie in the fact of difference in living standards, health facilities, over all hygienic condition and socio-economic structure of Auckland, New Zealand and Pakistan. An indigenous study by Rizwan et al., (2003), reported that control still faces major challenges. A total of 678 confirmed isolates of Mycobacterium Tuberculosis were subjected to anti Tuberculosis drug resistance. Five anti Tuberculosis drugs were studied for drug sensitivity pattern. The mono drugs resistance, which was highest for pyrazinamide 6% followed by rifampicin, isoniazid, ethambutol and streptomycin i.e. 4%,3.6%, 1.9% and 1.7% respectively.553% of isolates were resistant to one or more anti Tuberculosis drugs, resistance to rifampicin came out to be 28%, isoniazid 26%, streptomycin 24%, pyrazinamide 29%, ethambutol 15%, while resistance to MDR was 16%.5It is seen that Tuberculosis Control can be achieved with more focus on early case detection and prompt treatment. The global expansion of DOTS is now a days standard strategy for efficient control of Tuberculosis.

Poly resistance profile against 1st line anti-Tuberculosis drugs

Poly resistance profile of indigenous Mycobacterium Tuberculosis strains was studied on basis of resistance against two three or all of the four 1st line anti-Tuberculosis drugs have been studied. According to the results obtained, 20.35% Mycobacterium Tuberculosis strains resistant to any single drug, 9.30% strains resistant to any two anti-Tuberculosis drugs, 4.65% strains resistant to any three anti-Tuberculosis drugs and 4.07% strains resistant to all of the four 1stline anti-Tuberculosis drugs. 19.35% Mycobacterium Tuberculosis strains were resistant to rifampicin & isoniazid, 22.58% strains resistant to isoniazid & pyrazinamide, 3.22% strain resistant to ethambutol & pyrazinamide, 6.45% strains resistant to isoniazid & pyrazinamide, 22.58% strains resistant to rifampicin, isoniazid and pyrazinamide, 3.22% strain resistant to rifampicin, ethambutol and pyrazinamide and 22.58% strains resistant to all of the four 1stline drugs. 11.62% Mycobacterium Tuberculosis strains were multidrug resistant (minimally resistant to rifampicin and isoniazid). These finding are consistent with the findings of CCDR, (2004), who reported 12.5% resistance to one or more first line anti-tubercular drugs. 1.5% multi drugs resistance Tuberculosis (MDR-Tuberculosis) strains. The number of reported isolates in 2003 was relatively unchanged from the previous year (1420 in 2002 and 1379 in 2003 isolates).34With growing worldwide concern regarding Tuberculosis drug resistance, this surveillance system is vital in providing the necessary data in a timely fashion to monitor trend in Tuberculosis drug resistance in Canada. The reasons of difference of indigenous and Canadian tubercular resistance prevalence may lie in facts of excellent Canadian drug monitoring system, chemotherapeutic surveillance and an appreciable standard of health. Isik Johnsson et al., (1995), because of approximately similar pattern of resistance. While, Rizwan et at., (2003), reported the percentage of isolates resistant to two or more anti tubercular drugs along with comparison of primary and acquired drug resistance. The poly resistance profile of Mycobacterium Tuberculosis is as 14.6% (99) resistance to any two drugs, 8.11% (55) resistance to any three drugs, 5% (34) resistance to any four drugs and 4.71% (32) resistance to any five drugs. 5% of Tuberculosis patients were resistance to four drugs which is quite alarming. Hemvani et al., (2001), reported the resistance in India is higher to these drugs with exception to rifampicin 25% is comparable, MDR was 8.1% which is low as compared to 19 .4% of present study.The overall prevalence of drug resistance is 37% in Estonia, while in present study it is 38.37. The prevalence of MDR is 18% in Estonia which is comparable to 19.4% in present study. Moreover, Espinal et al., (2001), reported the overall prevalence of MDR is 18% in Estonia which is comparable to 19.4%in present study.5Some countries like Henan province in China, Latvia and Russia has MDR 10.8%, 9% and 9% respectively. While in Iran, Tomsk and Zhejiang province in China MDR is 5%,6.5% and 4.5%. However in France and United States MDR has decreased significantly. The possible difference may lie in difference of health facilities and socio- economic structure. WHO/ IUALTD, (2000), reported single anti-Tuberculosis drug resistance as 13.7% in china, 18.1% in Russian federation and 12.9% resistance in Estonia, resistance against any two anti-Tuberculosis drugs as 11.6% in china, 10.5% resistance in Russian Federation, 9.3% resistance in Estonia, resistance against any three anti-tubercular drugs as 5.6% resistance in India, 4.7% resistance in Russian Federation, 3.4% resistance in china and resistance against all of the four anti-Tuberculosis drugs observed in Iran as 3.7%, in china as 5.7%, in Russian federation as 6.2% and in china as 3%. The multidrug resistant cases were as 12.3% in Russian federation, 9.4% in china and 10.8% resistance in S. Mexico. Furthermore, Helen Heffernan (2002), studied surveillance of anti-Tuberculosis drug resistance based on the results of susceptibility testing of isolates in the Mycobacteriology Reference Laboratories at Auckland, Wellington and Waikato Hospitals. Antimicrobial susceptibility testing results17were available for all 268 isolates, which comprised 264 Mycobacterium Tuberculosis and four M. bovis isolates. There was 32 (11.9) resistant to any 1 agent, 11 (4.1) resistant to any 2 agents, 1 (0.4) resistant to any 3 agents, 1(0.4) resistant to all of the 4 agents. Patient infected with organism resistant to rifampicin have high rate of treatment failure when given the short course therapy (**Dikinson** & **Mitchison**, 1981). 5MDR Tuberculosis patient requires at least 18 months of therapy5or continues to have active Tuberculosis despite optimal therapy (Christopher 2002). The global burden of MDR Tuberculosis is difficult to estimate but the number of previously calculated cases of MDR Tuberculosis in the year 2000 is between 185000 and 414000 which is about 3% of all new Tuberculosis cases. Drug resistance is found to be in all the 72 countries surveyed in 2000.Particular hard bits are the Russian States, Eastern Europe and part of China according to these estimates Estonia is the MDR capital of the World with 14% of all new Tuberculosis cases being MDR Tuberculosis.In 1995, we reported increasing resistance rates to isoniazid and rifampin, four times higher than previously reported rates for Mexico (Sifuentes-Osornio et al., 2004). The Multi Drug Resistance Tuberculosis, resistance to at least isoniazid and rifampicin) is rare in New Zealand, with an average annual incidence of 0.7% (14 cases) recorded in the eight years since national surveillance of anti-Tuberculosis-drug resistance began in 1995. The possible reason of difference of obtained results may lie in the fact of big differences in living standards, health facilities, over all hygienic condition and socio-economic structure.

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Chapter 4

THERAPEUTICAL INTERPRETATION OF RESISTANT AGAINST 1ST LINE ANTI-TB DRUGS

The resistant Mycobacterium Tuberculosis having an ultimate highest level of resistance against the first line anti-Tuberculosis drugs - rifampicin, isoniazid, pyrazinamide and ethambutol, which are interpreted therapeutically to study the pharmacological suitability of dosage and regimen. The pharmacodynamic parameters - Cmax (maximum plasma concentrations), therapeutic index and standard dose, maximum regimens, unwanted effects etc considered for accurate scientific outcomes. Different combination of 1stline anti-Tuberculosis drugs used for miscellaneous time period; isoniazid, rifampicin and pyrazinamide for six months, isoniazid and rifampicin for nine months,68rifampicin, ethambutol and pyrazinamide for sixmonths, rifampicin and ethambutol for twelve months, isoniazid and ethambutol for eighteen months and others for two years (Richard et al., 2006). It is an exact evidence of effectiveness of lower plasma concentration of drugs used in combination therapy than standard MIC used in mono drug therapy. The standard dose given in single drug therapy is higher than the dose of same drug given in combination therapy. The drugs used in combination therapy act by different mechanisms to cure the disease. A similar study of reasoning the quantitative difference of doses required in individual and combination therapy was conducted by Stephen, 2002, who reported drug-resistant Tuberculosis poses a significant threat to human health and it is important to understand how the resistance emerges if we are to reverse the upward trend. Treatment with internationally approved regimens results in a very high cure rate with few relapses and without the emergence of resistance. These regimens are effective in preventing the emergence of resistance because combination chemotherapy makes it highly unlikely that there will be a spontaneous mutant resistant to all of the components of chemotherapy. Patients with uncomplicated Tuberculosis who receive inadequate treatment provide a selection advantage for resistant mutants because bacteria may be exposed to monotherapy, permitting the emergence of resistance to single agents and then to multiple agents as the protection of combination chemotherapy is eroded. That M. Tuberculosis cells within the body are susceptible to different components of anti-Tuberculosis chemotherapy means that the risk of resistant mutants emerging is higher than would be expected if the whole population of bacterial cells could be counted together.

Therapeutical interpretation of resistance against rifampicin

There were five rifampicin levels prepared in a local laboratory to determine the highest possible level of resistance of Mycobacterium Tuberculosis. It was observed that not` any rifampicin strain inhibited at 1st and 2nd drug levels. 40.54% resistant Mycobacterium Tuberculosis strains inhibited at 3rd rifampicin level of 120ug/ml. But, it is not feasible to maintain a plasma concentration higher than therapeutic range of 6.5±3.5ug/ml (Joel et al., 2001). 35.12% resistant Mycobacterium Tuberculosis were inhibited at 4 th rifampicin level 160 ug/ml, 18.91% inhibited at 5th rifampicin level 200 ug/ml and 5.40% inhibited at higher than 5th rifampicin level 200+ ug /ml. These final concentrations 160ug/ml and 200ug/ml incorporated in LJ media also exceed the therapeutic index. Therefore, can’t be used in actual clinical practice. If we exceed this therapeutic window or therapeutic index, it will produce sever unwanted effects of nausea, vomiting, rashes, hepatitis, GI upset, flu like syndrome and fever. Moreover, a sever jaundice may be introduced in chronic hepatic patient and alcoholic symptoms seen in elderly patients (Joel et. al., 2001). Patient is warned for red-orange in colored urine and tears. (Richared et al 2006). If these determined optimum concentrations decreased proportionately because of combination therapy with other anti-Tuberculosis drugs. Even then it exceeds the therapeutic range and can not be used in actual practices. The findings of this study are in line with the work of Richard et al., (2006), who reported the MIC as 0.005-0.2ug/ml, maximum plasma concentration (Cmax) 6 ±3.5 ug/ml, 98% plasma protein binding, 0.9L /Kg volume of distribution and standard therapeutical dose 600mg with maximum regimen of 1100mg per day. Our findings correlate with the work of Bertram, (2004), who reported 1.0ug/ml minimum inhibitory concentration, 5-7ug/ml maximum plasma concentration and 600mg daily dose of rifampicin. Our findings are agreement Venkatesan (1989), who reported the peak serum concentration of rifampicin for adults: 7 to 9 micrograms per mL (mcg/mL) after a single 600-mg oral dose and for children (6 to 58 months of age): Approximately 11 mcg/mL after a dose of 10 mg per kg of body weight (mg/kg). Rifampin tmax is 1.5 to 4 hours after oral administration; peak concentration may be decreased and delayed following administration with food. The volume of distribution is 1.6L per kg. The Protein binding of rifampin - high to very high (89%).15Rifampin crosses the placenta, with fetal serum concentrations at birth found to be approximately 33% of the maternal serum concentration; it penetrates into aqueous humor and is distributed into breast milk. Because it is lipid-soluble, rifampicin may reach and kill susceptible intracellular, as well as extracellular, bacteria and Mycobacterium species. These findings substantiate by the work of Dikinson & Mitchison, (1981), who reported the routine dosage of rifampicin 6mg/kg at a maximum regimen of 600mg per day. It is a generally well-tolerated drug, although it produces reddish coloring in the urine and other fluids, and it can cause hepatotoxicity. Another side effect is the appearance of a pseudo-flu syndrome. These results are consistent with the findings of Juan, (2006), who reported rifampicin oral or IV administration with bactericidal effect. Its daily dosage for adults is 10–20mg/kg and for children 8–12mg/kg. The maximum administrable regimen is 600mg. This drug acts on the rapid-growth populations of bacilli as well as on slow-multiplying populations. The findings of the study under discussion are substantiated by Taha et al., (2004), who reported rifampicin resistance >64ug/ml. These findings are in conformity with the work of CDCP, 2004, who reported MIC of rifampicin sensitive Mycobacterium Tuberculosis in the range of 1-50ug/ml. These findings are in agreement with the work of C Karin et al., 1997, Grim, 1978, Rizwan et al., 2003 and CCDR, 2003, who reported the optimum rifampicin concentration for declaration of sensitive or resistant Mycobacterium Tuberculosis as 40 ug/ml.

Therapeutical interpretation of resistance against isoniazid

There were five isoniazid levels - 0.2ug/ ml, 3ug/ml, 6ug/ml, 9ug/ml, 12ug/ml and 12+ ug/ml prepared to determine the highest possible level of resistance. It was observed that no any isoniazid strain inhibited at 1st, 2 nd and 3 rd drug levels. There 28% resistant Mycobacterium-Tuberculosis strains inhibited at 4 th isoniazid level 9ug/ml. Maximally plasma concentration that can be maintained in body is - 4ug/ml (Richard et al., 2006), therefore it can be used in actual practice. The maximum isoniazid regimen is 400mg (Leon Shargel et al, 2004). 24% resistant Mycobacterium Tuberculosis strains were inhibited at 5 th level of 12ug/ml, 48% resistant strains inhibited at higher than 5 th level of 12+ug/ml. These concentrations of 12ug/ml and 12+ug/ml incorporated in LJ media also exceed the therapeutic index. Therefore should not be maintained in actual tubercular treatment. If we exceed this therapeutic window or therapeutic index, the sever unwanted effects i.e. hypersensitivity, paresthenia, hepatic enzyme elevation, hepatitis and peripheral neuropathy, nausea, vomiting, epigastric distress, agranulocytosis, thrombocytopenia, fever, various rashes, dermatological and rheumatoid and lupoid syndromes may produce serious health hazards. The adverse effects are related to dosage and duration of administration. Paresthenia is the most common adverse effect, appears to be due to a relative pyridoxine deficiency. It is corrected by pyridoxine (vitamin B6) supplementation and therefore particularly recommended to lactating women to intake vitamin supplementation. The findings of the study under discussion substantiated by the work of Richard et al., 2006, who reported the standard MIC as 0.2-0.4ug/ml, plasma concentration 4ug/ml, maximally allowed plasma concentration 6ug/ml, the standard therapeutical dose 300mg with maximum regimen of 300mg. Our findings are in conformity with the work of Joel et al., (2001), who reported 0.2ug/ml MIC, 300mg standard dose, maximum plasma concentration in slow acetylation 5.4 ±2.0ug/ml and in fast acetylation 7.1±1.9ug/ml. These findings are in consistent with Holdiness, (1984), who reported the peak serum concentration of isoniazid 3 to 7 mcg/mL (21.9 to 51 micromoles per L) after a single 300-mg oral dose. 0.57 to 0.76 L per kg is the volum of distribution (Kergueris, 1983). Isoniazide is widely distributed to all fluids and tissues, including CSF, pleural and ascitic fluids, skin, sputum, saliva, lungs, and muscle. It crosses the placenta and is distributed into breast milk. The protein binding of isoniazid is very low (0 to 10%). These finding are in agreemetn with the findigns of Venkatesan D. (1989), who reported the time to peak serum concentration of isoniazid - 1 to 2 hours and peak plasma concentratioin (Cmax) as 4ug/ml. Findings of study under discussion substantiated by the findings of Juan M Broquetas Doñate, 2006, who reported oral or IM administered of isoniazid with bactericidal effect. Its daily dosage for children is 5–10mg/kg and for adult is 5mg/kg. The maximum daily administrable regimen is 300mg. These results are in consistent with the findings of WHO, (2001), who reported 100% minimum 11.11% median and 88.88% maximum resistance at 1stdrug level of 0.2 ug/ml and 100% minimum, 12.52% median and 87.5% maximum resistance at 5th drug level of 12 ug/ml. Finding of the study under discussion are substantiated by the findings of WHO/ IUALTD, (2000), who reported 58.33% resistance at 1stdrug level 0.2ug/ml, 8.33% resistance at 2nd drug level 3ug/ml and 33.33% resistance at 5 th drug level 12ug/ml. Our study is also supported the work of Jesudason et al., (2003), CCDR, (2004) and Victor et al., (1997), who reported the resistance of Mycobacterium Tuberculosis above of an optimum drug concentration of 0.2ug/ml.

Therapeutical interpretation of resistance against ethambutol

There were five ethambutol levels - 2ug/ ml, 4ug/ml, 6ug/ml, 8ug/ml and 10ug/ml prepared to determine the highest possible level of resistance. It was observed that no any ethambutol strain inhibited at 1stand 2 nd drug levels 2ug/ml and 4ug/ml. 50% resistant Mycobacterium Tuberculosis strains inhibited at 3 rd level of 6ug/ml. The maximum plasma concentration (Cmax) that can be maintained in Tuberculosis patient during treatment protocol are described by other researchers as 3-5ug/ml (Bertram, 2004), 2-5ug/ml (Leon et al., 2004) and 4-6ug/ml (Richard et al., 2006). It is very obvious from given data, exceeding the mentioned plasma concentration may introduce toxicity. The most important adverse effect of ethambutol is optic neuritis, which results in diminished visual acuity and loss of ability to discriminate between red and green (optic neuritis with blurred vision, red-green color blindness). Visual acuity should be periodically examined. Discontinuation of the drug results in reversal of the toxic symptoms. In addition, urate excretion is decreased by drug; thus gout may be exacerbated. (Richard, et al., 2006). Drug fever, abdominal pain, headache, dizziness and confusion also observed (Leon et al., 2004). 3(30%) ethambutol resistant Mycobacterium Tuberculosis was inhibited at 4th ethambutol level 8ug/ml, 2 (20%) ethambutol resistant strains inhibited at higher than 5 th level of 10ug /ml. The respective plasma concentration at 4th level of 8ug/ml and 5 th level of 10ug/ml can not be maintained without the risk of sever patient's health hazards. Therefore regimens are rejected to use in actual chemotherapeutical practice. The findings of this study are in line with the work of Richard et al., 2006, who reported 1-8ug/ml minimum inhibitory concentration (MIC), 4-6ug/ml maximum plasma concentration (Cmax) and 6ug/ml maximally permitted plasma concentration. Our findings also correlate with the work Joel et al., (2001), who reported minimum inhibitory concentration 1- 5ug/ml, maximum plasma concentration (Cmax) 2-5ug/ml, standard dose 400mg or 25mg/kg/day and maximally regimen 1100mg. Similar findings reported by Myambutol (Lederle), (2000), that depicts the peak serum concentration as 2 to 5 mcg/mL after a single dose of 25 mg/kg with the time to peak serum concentration of 2 to 4 hours. The protein binding of ethambutol is low (20 to 30%). Distribution of ethambutol is to most tissues and body fluids, except CSF. Ethambutol does not penetrate intact meninges, but 10 to 50% may penetrate the meninges of patients with tubercular meningitis. These findings are also in conformity with Venkatesan et al (1998), who reported volume of distribution 1.6 L per kg. Findings of study under discussion are substantiated by the findings of Juan, (2006), who reported daily ethambutol dosage 15–25mg/kg and the maximum administrable per day regimen 2.5g. The main function of ethambutol is to prevent the appearance of resistance. It is generally well-tolerated and has few toxic effects. There appears to be a possibility of retrobulbar neuritis. It is therefore not recommended for children who are unable to receive frequent ophthalmological check-ups. The findings of this study are in line with the work of WHO/ IUALTD, (2000), who reported 75% resistance at 1stdrug leval., 12.5% resistance at 2 nd drugs level and 12.5% resistance at 4 th drug level. These finding are in conformity with the work of CCDR, (2004), who reported the resistance of ethambutol resistance Mycobacterium Tuberculosis above than the critical concentration of 2ug/ ml.

Therapeutical interpretation of resistance against pyrazinamide

There were five pyrazinamide levels - 100ug/ml, 200ug/ml, 300ug/ml, 400ug/ml and 500ug/ml prepared to determine the highest possible level of resistance. It was observed that no any pyrazinamide resistant strain inhibited at 1stand 2nd drug levels100ug/ml and 200ug/ml. 27.66% pyrazinamide resistant Mycobacterium Tuberculosis strains were inhibited at 3 rd pyrazinamide level of 300ug /ml. The maximum plasma concentration than can be maintained in human body reported by different researchers are 9-12ug/ml (Joel et al, 2001), 19ug/ml (Leon et al., 2004), 30-50ug/ml (Bertram, 2004), 37-40ug/ml (Richard et al., 2006). Exceeding of this maximally permitted plasma concentration may introduce sever health hazards. Maintenance of aforesaid plasma concentration is therefore rejected to use in actual chemotherapeutical practice. During the pyrazinamide toxicity the important adverse effect introduced by pyrazinamide are the nausea, hyperuricemia, rashes, joint ache, gout (rare) (Richard et al., 2006), fever, anorexia, malaise, and hepatitis with or without hepatic jaundice, and death can occur (Alfonso et al., 1998). 25.53% resistant Mycobacterium Tuberculosis were inhibited at 4th pyrazinamide level 400ug/ml, 31.91% resistant strains inhibited at 5 th pyrazinamide level 500ug/ml and 14.89% pyrazinamide resistant strains inhibited at higher than 5th pyrazinamide level 500+ug /ml. The respective plasma concentration at 5th level of 500ug/ml and higher than 5 th level of 500+ug/ml can't be maintained in Tuberculosis patient without the risk of sever patient's health hazards. Therefore regimens are rejected to use in actual chemotherapeutical practice. The findings of this study are in line with the work of Richard et al., (2006), who reported 18-23ug/ml minimum inhibitory concentration, 37-40ug/ml maximum plasma concentration, 500mg standard therapeutical dose with maximum regimen of 800mg. Our findings are consistent by the work of Taha et al., (2004), who reported the maximum plasma concentration (Cmax) 40ug/ml, critical drug concentration that incorporated in LJ media to consider as border line for declaration of sensitive or resistant bacteria 100ug/ml, standard dose 500mg and maximally permitted dose 800mg. Our finding are in line with the work of Bertram et al., (2004) who reported the minimum inhibitory concentration 100ug/ml, maximum plasma concentration 30-50ug/ml and standard dose of 25mg/kg/day. These findings are in conformity with Juan, (2006), who reported the usual pyrazinamide dosage 25mg/kg/day with a maximum of 2g per day. Pyrazinamide administered orally and having the bactericidal effect. Its daily dosage for children 20–30 and for adult is 1.5g <50kg 2.0g (51–74kg) 2.5g >75kg. Thrice weekly dose for adult is 2g <50kg 2.5g (51–74kg) 3.0g >75kgl. The action of pyrazinamide is fundamental on slow-multiplying and intercellular bacilli and has a sterilizing effect. It is hepatoxic and interferes with the metabolism of uric acid by increasing its level. The Volum of distribution of pyrazinamide is 0.57 to 0.74 L per kg (Lacroix et al., 1989). These findigns are in line with Ellard, (1987), who reported widely distribution of pyrazinamide to most fluids and tissues, including liver, lungs, kidneys, and bile. Pyrazinamide has excellent penetration into CSF, ranging from 87 to 105% of the corresponding serum concentration. These findings are in agreement with Stamatakis et al., (1988), who reported the plasma protein binding of pyrazinamide low (10 to 20%), the time to peak serum concentration 1 to 2 hours and the peak serum concentration of approximately 19 mcg/mL after a single dose of 14 mg/kg and approximately 39 mcg/mL after a single dose of 27 mg/kg (Lacroix et al., 1989). The findings of this study are in line with the work of Taha et al., (2015), who reported the final concentration (100ug/ml) incorporated in LJ media to consider as border line to declare the sensitive or resistant Mycobacterium Tuberculosis was very much clearly higher than the 100ug/ml. Study by Joel et al., (2001), also reveal a similar findings of 12.5 minimum inhibitory concentration, 9- 12ug/ml maximum plasma concentration following a regimen of 500mg per day.

Resistance trend against 1st line anti-Tuberculosis drugs during Nov. 2004 to Dec. 2005.

The Mycobacterium Tuberculosis strains studied for evaluation of overall trend of resistance prevalence against all of the four 1stline anti-Tuberculosis drugs, during Nov. 2004 to Dec. 2005. The total Mycobacterium Tuberculosis strains were chronologically compared over number of resistant drugs. There was a gradual decline observed in resistance profile of Mycobacterium Tuberculosis from January to December, 2005. These findings are in consistent with the work of WHO/ IUALTD, (2000), who reported a decrease in overall resistant prevalence against Mycobacterium Tuberculosis. Finding of study under discussion substantiated by the findings of CCDR, (2004), who reported the percentage of isolates demonstrating any type of drug resistance was unchanged between the two reporting years (12.6% in 2002 and 12.5% in 2003), and proportion of Multi Drug Resistance tuberculois was identical (1.5%) in both years. Overall, level of Tuberculosis drug resistance has shown no significant difference since inception of this reporting system in 1998. Study by Rizwan et al., (2003), revealed the comparison of drug resistance for MDR and other anti-Tuberculosis drugs for the year 1998 to 2002. It is evident from this that MDR and resistance to other drugs are on increase with the passage of time. The possible reason may lie in the fact of gape of approximately five years that made the increasing trend of resistance static. A number of lessons for future efforts to control Tuberculosis in Pakistan could be derived from experiences of other countries one and only successful strategy is rapid implementation and expansion of DOTS, being the only solution to an alarming increase of drug resistant Tuberculosis in Pakistan. Our finding are consistent with Xianyi, (2002), who reported the implementation of DOTS rapidly on large scale in different countries has demonstrated that global target of 85% cure rate was quickly achieved, and the level of drug resistance was probably reduced by these projects. Our findings are in agreement with Helen (2002), who reported similar findings in 2002. Antimicrobial susceptibility testing results were available for all 268 isolates, which comprised 264 Mycobacterium Tuberculosis and four M. bovis isolates.17Over the last eight years, there has been a trend of increasing streptomycin resistance ($p=0.0078$). Isoniazid and pyrazinamide resistance has fluctuated, while rifampicin and ethambutol resistance has been relatively stable and remained ≤2%. There some differences observed in resistance profile against 1stline anti-Tuberculosis drugs. The possible reason may lie in the fact of big differences in living standards, health facilities, over all hygienic condition and socio-economic structure. There also involvement of an estimate of drug resistance is extremely important in the epidemiology and control of Tuberculosis (Hemvani, 2001). World wide accepted DOTS strategy seems on answer to rapid emergence of anti Tuberculosis drug resistance. A number of projects for future efforts to control Tuberculosis in China and other countries can be derived from the results of 10 years experience in DOTS expansion and implementation. In less than five years, DOTS coverage expanded from zero to more than 90% of target areas and population (Xianyi, 2002).

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Chapter 5

INDIGENOUS PATTERN OF RESISTANCE OF MYCOBACTERIUM Tuberculosis IN PAKISTAN

Prevalence of Tuberculosis is an alarming issue for developing nations of the world. The halves of all new TB cases are noticed in Bangladesh, India, Pakistan, Indonesia and Philippine (Bitar et al., 2001). Particularly the resistance of *Mycobacterium Tuberculosis* (*M. Tuberculosis*) against fist line anti-Tuberculosis drugs; rifampicin, isoniazid, ethambutol and pyrazinamid is an issue of great importance. Furthermore; certain biological and clinical factors are constantly contributing to develop the resistance. It is induced by poor therapeutical surveillance (CCDR, 2004), substandard socioeconomic condition, inaccurate regimens and genetic mutations (Christopher, 2002). However, an optimal regimen and duration of the treatment remains a matter of conversation. (Isik et al., 2002)

There was also a research project conducted to study the pattern of development of resistance of *M. Tuberculosis* against 1st line anti-tubercular drugs; rifampicin, isoniazide, ethambutol and pyrazinamid. (Espinal et al., 2001)That may help to assess the clinical credibility of prevailing treatment programs, protocols and schemes. The pure chemical of these drugs were obtained from the Schazoo Laboratories (Pvt.), GT Road, Lahore, Pakistan.

Lowenstein Jensen (LJ) Medium

Lowenstein Jensen medium was prepared by the method described by Nazir *et al.* (2008) and used for primary culturing of the processed samples. The LJ medium slants were inoculated with processed samples in class-II safety cabinet (Telstar, Spain). The growth of *M. Tuberculosis* thus obtained was identified though Acid Fast staining and further used for sensitivity testing.

Table 1. Preparation of five different dilutions / proportions of rifampicin, isoniazid, pyrazinamid and ethambutol inoculated in LJ-media for determination of level of resistance of resistant M-TB.

	RFP μg/ ml	**ISN** μg/ ml	**PZA** μg/ ml	**ETH** μg/ ml
MIC (μg/ ml)	40	0.2	100	2
1st Dilution	80	3	200	4
2nd Dilution	120	6	300	6
3rd Dilution	160	9	400	8
4th Dilution	200	12	500	10
5th Dilution	200+	12+	500+	10+

RFP- Rifampicin ETB- Ethambutol PZA- Pyrazinamid
ISN-Isoniazid MIC- minimum inhibitory concentration

Preparation of inoculums of *Mycobacterium Tuberculosis*

The over seeding of a drug containing medium was prevented by standardization of inoculums that mitigate the possibility of miss judgment of considering the resistance to a susceptible strain. The representative samples of growth containing minimally 50 colonies were taken from primary culture and placed into McCartney vials containing 1mL of distilled water and 5 glass beads. The mixtures were homogenized by vigorous stirring by Vortex Mixer (Eyela, Japan) for 1-3 minutes and left in class-II safety cabinet (Telstar, Spain). The detailed process of preparation of inoculums and testing the susceptibility of *M. Tuberculosis* is explained by Taha et. al., (2010).

Data interpretation

The Bijoux bottles were inspected weekly for appearance of growth. Number and morphology of colonies were noted as growth was evident on LJ medium. The cultures with no growth were discarded after 8 weeks of incubation. The results were interpreted for resistance on the basis of percentage of colonies on drug containing media in comparison to the growth on drug free medium.

The clinical isolates of sputum (84.9%), pus (10.5%) and bronchial washings (4.7%) were collected from Tuberculosis diagnosed patients. These specimens comprised of (84.3%) pulmonary, (15.7%) extra-pulmonary, (70.9%) males and (29.1%) females patients.

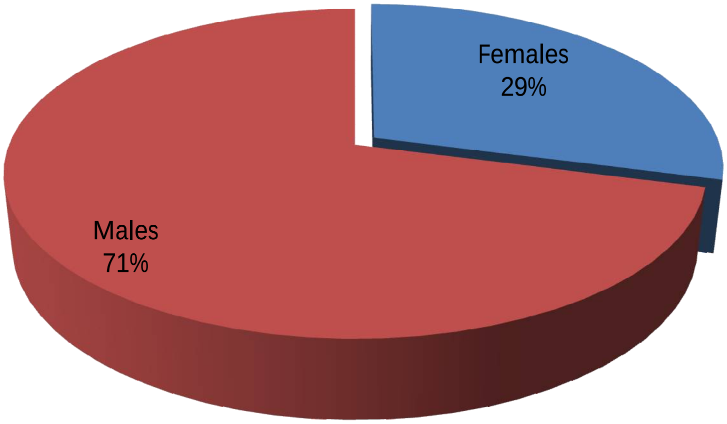

Figure: Comparison of male and female Tuberculosis positive (AFB positive) patients.

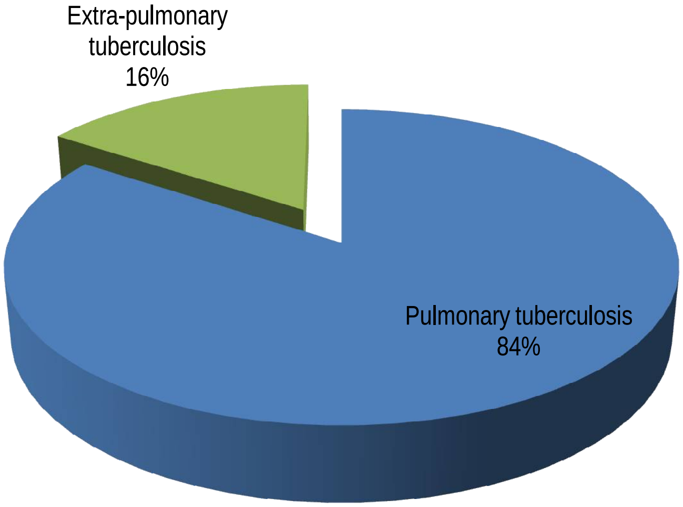

Figure: Distribution of the biological samples collected form TB suspicious patients.

Bitar *et al.* (2001) reported the pulmonary TB cases 89% (n=607), extra-pulmonary Tuberculosis 6%(n=39) and unknown site patients 5%(n=37) cases. Uplekar *et al. (2001)* reported a seventy percent (70%) excess of males over females globally each year. Haq *et al.* (2002) reported 68% male and 32% female Tuberculosis patients. WHO/ IUALTD (2000) reported 67% of male Tuberculosis patients.

Mono and poly drug resistance of *Mycobacterium TB*

The mono drug resistance of *M. Tuberculosis* was observed as 25.71% clinical isolates resistant to rifampicin, 8.57% to isoniazid, 2.85% to ethambutol and 62.85% to pyrazinamide out of (20.34% mono-resistant strains of total samples.

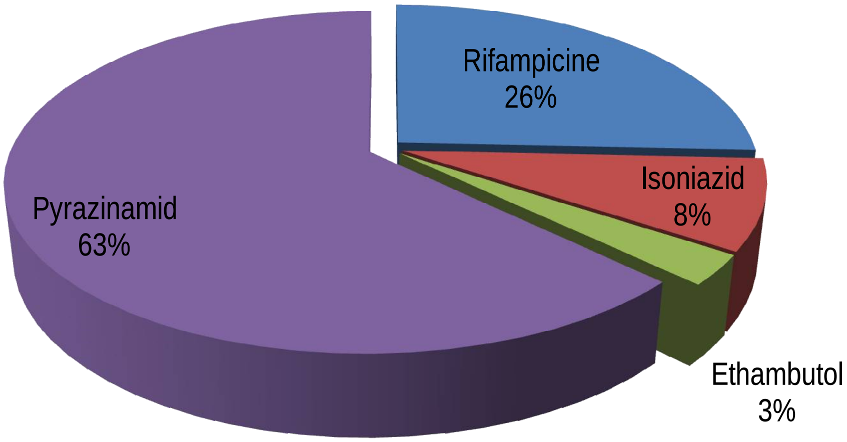

Figure. The resistance pattern of indigenous *Mycobacterium Tuberculosis* strains against 1st line chemotherapy collected from primary culture of Tuberculosis diagnosed (AFB positive) patients of 14-67 years age group.

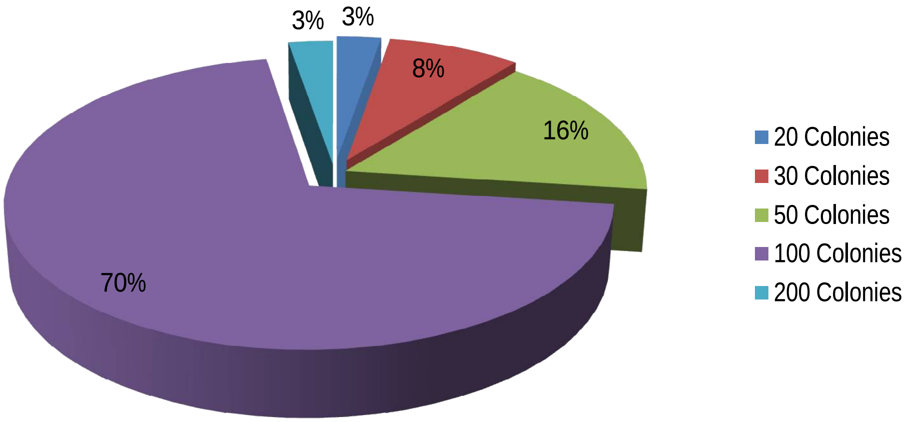

Figure: Comparison of resistance percentage and quantity of growth/ number of colonies of indigenous *Mycobacterium Tuberculosis* in rifampicin incorporated Lowenstein Jensen media, collected from primary culture of Tuberculosis diagnosed (AFB positive) patients of age 17-68 years.

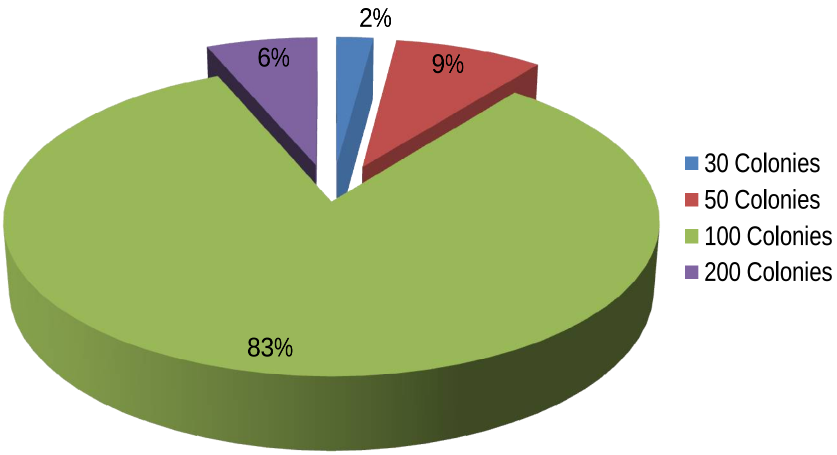

Figure: Comparison of resistance pattern (percentage) and quantity of growth of indigenous *Mycobacterium Tuberculosis* in pyrazinamide incorporated Lowenstein Jensen media

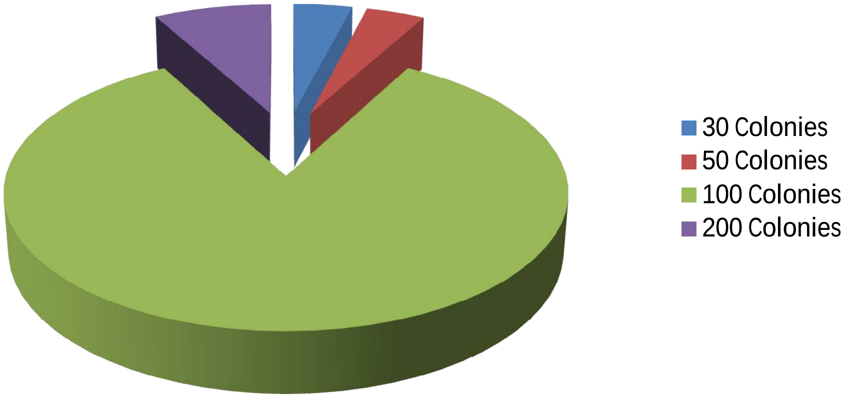

Figure: Comparison of resistance pattern (percentage) and quantity of growth of indigenous *Mycobacterium Tuberculosis* in isoniazid incorporated Lowenstein Jensen media.

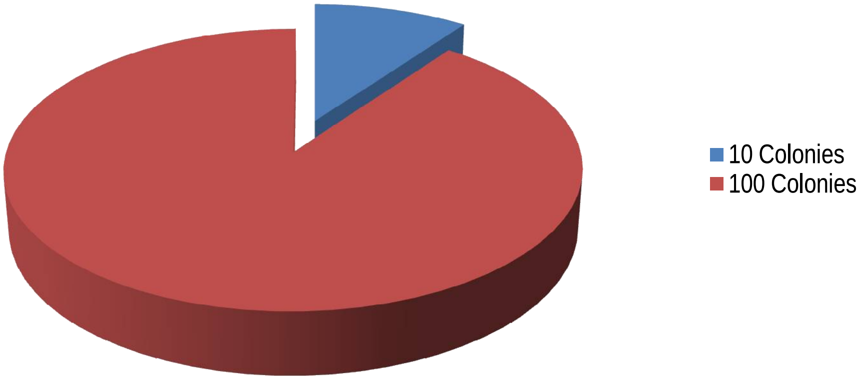

Figure: Comparison of resistance pattern (percentage) and quantity of growth of indigenous *Mycobacterium Tuberculosis* in ethambutol incorporated Lowenstein Jensen media.

Helen, (2002), who reported similar findings; resistant to isoniazid 9.7%, rifampicin 1.5%, ethambutol 1.9% and pyrazinamide 4.1%. There significant differences observed in resistance profile of rifampicin and pyrazinamide. The possible reasons may lie in the fact of difference in living standards, health facilities, over all hygienic condition and socio-economic structure of Auckland, New Zealand and Pakistan. Whereas, Rizwan *et al.,* (2003), reported the mono drugs resistances against pyrazinamide, rifampicin, isoniazid, ethambutol and streptomycin as 6%, 4%, 3.6%, 1.9% and 1.7% respectively. 53% of clinical isolates were resistant to one or more anti Tuberculosis drugs, resistance to rifampicin came out to be 28%, isoniazid 26%, streptomycin 24%, pyrazinamide 29%, ethambutol 15%, while resistance to MDR was 16%. It is seen that TB Control can be achieved with more focus on early case detection and prompt treatment. The global expansion of DOTS is now a day's standard strategy for efficient control of TB.

Poly drug resistance of *Mycobacterium Tuberculosis*

Poly resistance profile of indigenous strains was obtained as 19.35% resistant to rifampicin & isoniazid, 22.58% to ethambutol & pyrazinamide, 3.22% to ethambutol & pyrazinamide, 6.45% to isoniazid & pyrazinamide, 22.58% to rifampicin, isoniazid and pyrazinamide, 3.22% to rifampicin, ethambutol and pyrazinamide and 22.58% to all of the four 1st line drugs.

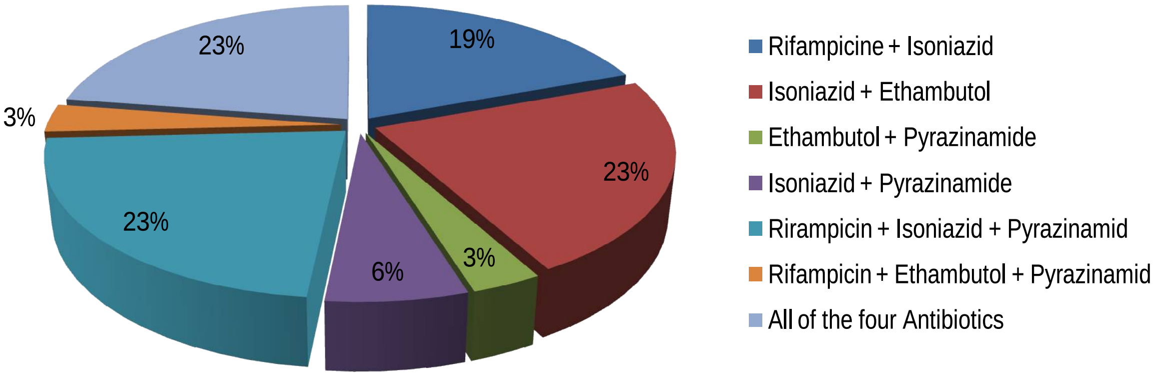

Figure: Poly-resistance profile of indigenous pulmonary/ extra-pulmonary *Mycobacterium TB* strains categorized on basis of resistance against one, two, three or all of the four 1st line antitubercular drugs.

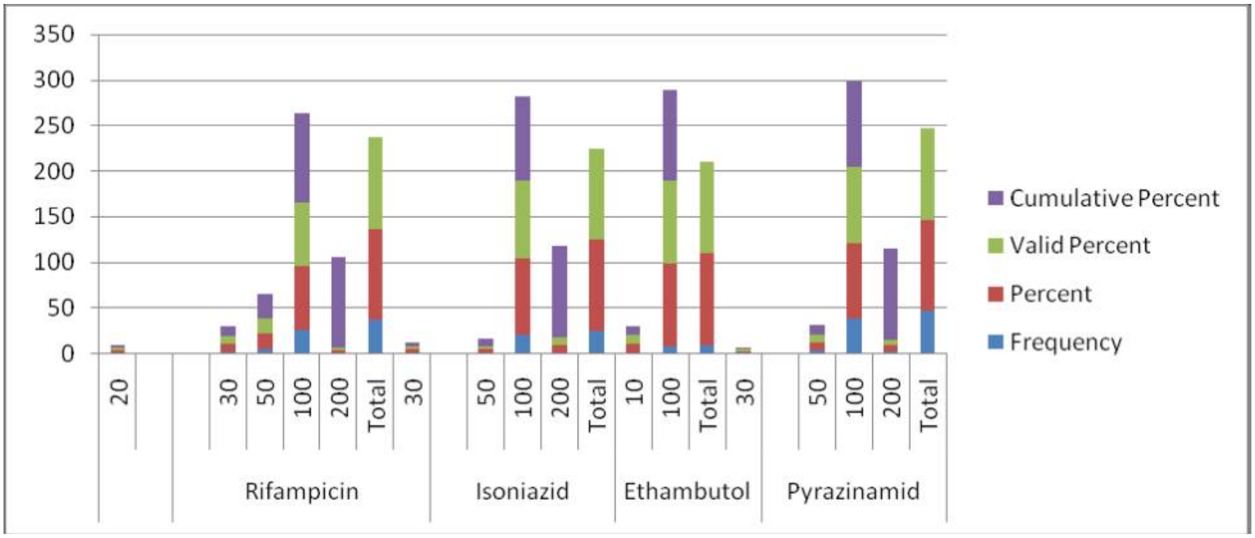

Figure: Comparative resistance pattern of M. Tuberculosis against rifampicin, isoniazid, ethambutol and pyrazinamid

Poly resistance profile of *Mycobacterium Tuberculosis* strains was also studied on basis of resistance against one, two, three or all of the four 1st line anti-Tuberculosis drugs. Data showed 20.35% strains resistant against one drug, 9.30% against two drugs, 4.65% against three drugs and 4.07% against all of the four anti-TB drugs, while 61.63% strains were susceptible.

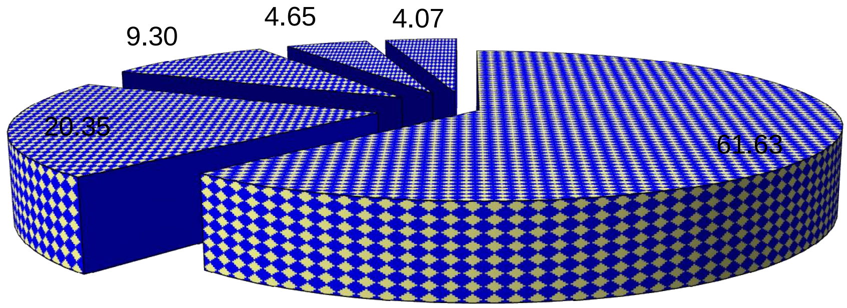

Figure: Poly-resistance profile (% age) of indigenous pulmonary/ extrapulmonary *Mycobacterium TB* on basis of number of drugs to be resistant against.

Our findings are in consistent with CCDR, (2004), who reported 12.5% resistance to one or more first line anti-tubercular drugs. 1.5% multi drugs resistance Tuberculosis (MDR-TB) strains. The numbers of reported isolates in 2003 were relatively unchanged from the previous year (1420 in 2002 and 1379 in 2003 isolates). With growing worldwide concern regarding TB drug resistance, this surveillance system is vital in providing the necessary data in a timely fashion to monitor trend in TB drug resistance in Canada. The reasons of difference of indigenous and Canadian tubercular resistance prevalence may lie in facts of excellent Canadian drug monitoring system, chemotherapeutic surveillance and an appreciable standard of health. Isik Johnsson *et al.,* (1995) reported approximately similar pattern of resistance. Rizwan et at., (2003) reported the poly resistance profile as 14.6% (99) resistance to any two drugs, 8.11% (55) resistance to any three drugs, 5% (34) resistance to any four drugs and 4.71% (32) resistance to any five drugs. Hemvani *et al.,* (2001) reported the higher resistance in India with the exception of rifampicin 25% that is comparable. MDR were 8.1% that is low as compared to 19.4% of present study. Espinal *et al.,* (2001) reported the 18% overall prevalence of MDR in Estonia that is comparable with 19.4% of present study. WHO/ IUALTD, (2000) reported single drug resistance as 13.7% in china, 18.1% in Russian federation and 12.9% resistance in Estonia, two drugs resistance as 11.6% in china, 10.5% resistance in Russian Federation, 9.3% resistance in Estonia, three drugs resistance as 5.6% in India, 4.7% in Russian Federation, 3.4% in china and all of the four drugs resistance as 3.7% in Iran as, 5.7% in china, 6.2% in Russian federation and 3% in china. The multidrug resistant cases were as 12.3% in Russian federation, 9.4% in china and 10.8% in S. Mexico. Helen Heffernan (2002) reported surveillance of drug resistance based on the results of susceptibility testing of Mycobacteriology Reference Laboratories at Auckland, Wellington and Waikato Hospitals. There were 32 (11.9%) strains resistant to 1 agent, 11 (4.1%) resistant to 2 agents, 1 (0.4%) resistant to 3 agents, 1 (0.4%) resistant to all of the 4 agents. Patient infected with organism resistant to rifampicin have high rate of treatment failure when given the short course therapy (Mitchison & Nuna, 1986). MDR TB patient requires at least 18 months of therapy or continues to have active TB despite optimal therapy (Christopher 2002). Drug resistance is found to be in all the 72 countries surveyed in 2000. Particular hard bits are the Russian States, Eastern Europe and part of China according to these estimates Estonia is the MDR capital of the World with 14% of all new TB cases being MDR TB. Sifuentes-Osornio J *et al.,* (1995) reported increasing resistance rates against isoniazid and rifampin is four times higher than previously reported rates for Mexico. The possible reason of difference in obtained results of resistance may lie in the fact of big differences in living standards, health facilities, hygienic condition and socio-economic structure.

The highest risk of mono-drug resistance noticed against pyrazinamid and least potential of resistance observed against ethambutol. The poly-resistance pattern pointed out highest potential of resistance against isoniazid & ethambutol and least against ethambutol & pyrazinamid in two drugs category; the maximum resistance potential seen against rifampicin, isoniazid & ethambutol and least against rifampicin, ethambutol & pyrazinamid in three drugs category. Thus; the combinations with least resistance potential should be preferred in actual clinical practice to avoid the development of resistance and assure the accomplishment of TB control programs.

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Chapter 6.

TREND OF RESISTANCE OF *Tuberculosis* AGAINST FIRST LINE ANTI-TUBERCULAR DRUGS

Tuberculosis is the world's greatest infectious killer of women of reproductive age and the leading cause of death among people with HIV/ AIDS (Koh et al., 2005). Roughly a third of the world's population is infected with *Tuberculosis* and new a patient is introduced after each second (Nolan & Goldberg, 2002); 13.7 million with active TB, 9.3 million new enrolments, 1.8 million deaths along with annual incidence rate of 363 per 100,000 in Africa to 32 per 100,000 in the America (WHO, 2005). Thus we have compiled this book to describe the trend of resistance of *Tuberculosis* against 1st line anti-Tuberculosis drugs.

The emergence of resistance of Mycobacterium Tuberculosis against 1st line antitubercular drugs is alarming the problem of great importance to public health. Some of the clinical, biological and epidemiological factors are constantly contributing to develop resistance. It is also induced by inaccurate treatment protocols, substandard socioeconomic condition and poor therapeutical surveillance (Herendra & Shah, 1998) and genetic mutations (Barnes et al., 1991). The recent increase in the incidence of this disease and emergence of resistance has urged the need to study the trend of resistance.

The half of all new TB cases are in 6 Asian countries – Bangladesh, India, Pakistan, Indonesia and Philippine and multidrug resistance (isoniazid and rifampicin resistance with/ without resistance against other anti-TB drugs) present virtually all 109 countries (Taha et al., 2008). In most developing countries, the disease has always been endemic (Bitar et al., 2001) but in industrialized countries the development of resistance, suppressed immunity and contributed the disease's resurgence (Uplekar et al., 2001). However, an optimal regimen and duration for this treatment remains a matter of some controversy. Here, we tried to share our experience in a case of the primary and acquired resistance. The major objective of our work is to study the trend of resistance of *M. Tuberculosis* against rifampcin, isoniazid, ethambutol and pyrazinamide to decide the clinical credibitily of Tuberculosis treatment programs.

There was a study conducted in Pakistan to investigate the trend of resistance of M. Tuberculosis against rifampicin, isoniazide, ethambutol and pyrazinamid. The pure chemical of these drugs were provided by Schazoo Laboratories (Pvt.), Lahore, Pakistan. The pulmonary and extra-pulmonary Tuberculosis diagnosed (AFB positive) patients were selected from six different sources. The patients of all age groups were selected, regardless of their age, gender and previous therapeutic profile. The sputum, pus and bronchial washings were collected, labeled and stored, separately. All the samples were centrifuged decontaminated and processed as described by Taha *et. al.,* (2008). Lowenstein Jensen medium was prepared by the method described by Nazir *et al.* (2010) and used for primary culturing of the processed samples. The LJ medium slants were inoculated with processed samples in class-II safety cabinet (Telstar, Spain). The growth of *M. Tuberculosis* thus obtained was identified though Acid Fast staining and further used for sensitivity testing.

Table 1: The final concentration of antitubercular agents incorporated in LJ media.

#	Drug	Concentration
1	Rifampicin	40 µg/ ml
2	Isoniazid	0.2 µg/ ml
3	Pyrazinamide	100 µg/ml
4	Ethambutol	2 µg/ ml

The over seeding of a drug containing medium was prevented by standardization of inoculums that mitigate the possibility of miss judgment of considering the resistance to a susceptible strain. The representative samples of growth containing minimally 50 colonies were taken from primary culture and placed into McCartney vials containing 1mL of distilled water and 5 glass beads. The mixtures were homogenized by vigorous stirring by Vortex Mixer (Eyela, Japan) for 1-3 minutes and left in class-II safety cabinet (Telstar, Spain). The detailed process of preparation of inoculums and testing the susceptibility of *M. Tuberculosis* is explained by Taha et. al., (2015).

Recording and interpretation of results

The Bijoux bottles were inspected weekly for appearance of growth. When the growth was evident on LJ medium, colony morphology was noted. One culture bottle was exposed to day light for one hour and re-incubated to be examined for pigmentation on the following day. The cultures with no growth were discarded after 8 weeks of incubation. The presence and amount of growth in term of number of colonies on control and drug inoculated medium recorded. The results were interpreted for resistance on the basis of percentage of colonies on drug containing media in comparison to the growth on drug free medium. The strains showing susceptibility were again incubated and examined at 6 weeks before declaring as sensitive. The growth pattern, number of colonies and contamination were checked carefully on weekly basis

The *Mycobacterium TB* strains were studied for evaluation of overall trend of resistance prevalence against all of the four 1st line antitubercular drugs. *Mycobacterium TB* strains were divided into four segments (each segments comprised of 43 *Mycobacterium TB* strains). In each segment the strains were chronologically studied for resistance against drugs. In 1st segment 11 strains were resistant to 1 drug, 6 to 2 drugs, 3 to 3 drugs and 4 to the entire of the four drug (only 20 strains were found sensitive to all of the four drugs). In 2nd segment 11 strains were resistant to 1 drug, 4 to 2 drugs, 5 to 3 drugs and 4 to the entire 4 of the drug (only 19 strains were found sensitive to all of the four drugs). In 3rd segment there were 9 strains were resistant to 1 drug and 3 strains to 2 drugs (only 31 strains were sensitive to all of the four drugs). In 4th segment 4 strains were resistant to 1 drug and 3 to 2 drugs (only 36 strains were found sensitive to all of the four drugs) (Figure 1).

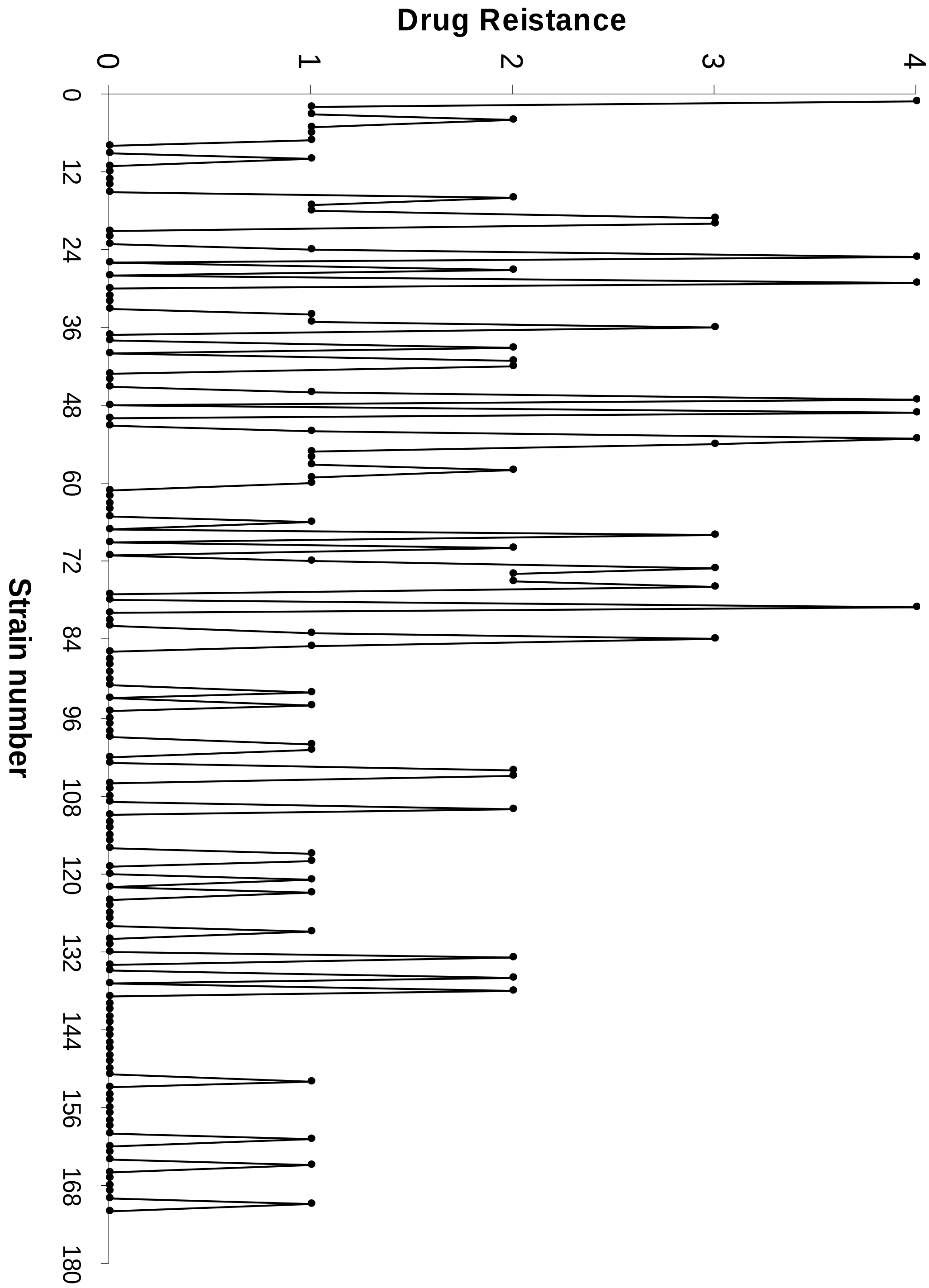

Fig.43. Overall trend of resistance prevalence of indigenous Mycobacterium -*TB* during November, 2004-December, 2005.

The number of *M. Tuberculosis* strains and their resistant pattern against drugs was studied chronologically. There was a gradual decline observed in resistance profile of *Mycobacterium Tuberculosis* from November, 2004 to January, 2006. These findings are in consistent with the work of WHO/ IUALTD, (2000), who reported a decrease in overall resistant prevalence against *Mycobacterium Tuberculosis*. Finding of study under discussion substantiated by the findings of CCDR, (2004), who reported the percentage of isolates demonstrating any type of drug resistance was unchanged between the two reporting years (12.6% in 2002 and 12.5% in 2003), and proportion of MDR-TB was identical (1.5%) in both years. Overall, level of TB drug resistance has shown no significant difference since inception of this reporting system in 1998. Study by Rizwan *et al.,* (2003), revealed the comparison of drug resistance for MDR and other anti tubercular drugs for the year 1998 to 2002. It is evident from this that MDR and resistance to other drugs are on increase with the passage of time. The possible reason may lie in the fact of gape of approximately five years that made the increasing trend of resistance static. A number of lessons for future efforts to control TB in Pakistan could be derived from experiences of other countries one and only successful strategy is rapid implementation and expansion of DOTS, being the only solution to an alarming increase of drug resistant TB in Pakistan. Our finding are consistent with Xianyi, (2002), who reported the implementation of DOTS rapidly on large scale in different countries has demonstrated that global target of 85% cure rate was quickly achieved, and the level of drug resistance was probably reduced by these projects. Our findings are in agreement with Helen Heffernan (2002), who reported similar findings in 2002. Antimicrobial susceptibility testing results were available for all 268 isolates, which comprised 264 *Mycobacterium Tuberculosis* and four *M. bovis* isolates. Over the last eight years, there has been a trend of increasing streptomycin resistance (p=0.0078). Isoniazid and pyrazinamide resistance has fluctuated, while rifampicin and ethambutol resistance has been relatively stable and remained ≤2%. There some differences observed in resistance profile against 1st line antitubercular drugs. The possible reason may lie in the fact of big differences in living standards, health facilities, over all hygienic condition and socio-economic structure. There also involvement of an estimate of drug resistance is extremely important in the epidemiology and control of Tuberculosis (Hemvani, 2001). World wide accepted DOTS strategy seems on answer to rapid emergence of anti TB drug resistance. A number of projects for future efforts to control TB in China and other countries can be derived from the results of 10 years experience in DOTS expansion and implementation. In less than five years, DOTS coverage expanded from zero to more than 90% of target areas and population (Xianyi, 2002).

In conclusion; a gradual decline in overall resistance of *Mycobacterium Tuberculosis* was observed. The Tuberculosis control programs are seemed to be successful to eradicate the development of resistance. The study also guide us for future efforts to control resistance in this area. It is also derived from the experiences that the successful strategies of rapid implementation of treatment protocols are effective in mitigate mortality. The only solution to an alarming increase of drug resistant is the standardized therapy and continuous follow up.

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HIGHLIGHTS

On basis of present study, the following conclusions are drawn,

1. Most tubercular patients founded in wards. They were seriously ill and required special attentions. These indoor departments are also observed as the source of contagious and nosocomial tubercular infection.
2. The sputum is most accepted specimen used to diagnose the Tuberculosis. It is also used to determine the sensitivity of pulmonary Mycobacterium Tuberculosis. Bronchial washing, puss and biopsy fluid also used to evaluate certain clinical tests.
3. Epidemiological comparison of pulmonary and extra-pulmonary Tuberculosis pointed out the five time higher prevalence of pulmonary Tuberculosis than extra-pulmonary Tuberculosis.
4. A significant difference was observed among gender distribution of Tuberculosis. Male tubercular patient were four time higher than female patients.
5. The pattern of resistance of indigenous Mycobacterium Tuberculosis against rifampicin was resolute by drug proportion technique using Lowenstein Jensen medium. Approximately 1/5 of the total collected Mycobacterium Tuberculosis stains were founded resistant to rifampicin.
6. Approximately 1/6 of total collected Mycobacterium Tuberculosis strains were resistant to isoniazid. It is also the primary anti-Tuberculosis drug that contributes to declare as multidrug resistant Tuberculosis.
7. Approximately 1/16 of total Mycobacterium Tuberculosis strains were resistant to ethambutol. It is the minimum number of Mycobacterium among all of the four examined anti-Tuberculosis drugs.
8. Approximately ¼ of total collected Mycobacterium Tuberculosis strains were resistant to pyrazinamide. It is the maximum resistance of Mycobacterium-Tuberculosis among all of the four examined anti-Tuberculosis drugs.
9. Maximum monodrug resistance observed against pyrazinamide and minimum resistance against ethambutol concluded by the overall comparison of mono- resistances of Mycobacterium Tuberculosis strains against all the 1stline anti- tubercular drugs.
10. Three categories of poly-resistant Mycobacterium Tuberculosis were observed on basis of resistance against two, three and all of the four anti-Tuberculosis drugs. In two drugs category, isoniazid & ethambutol having the maximum and ethambutol & pyrazinamide having the minimum resistance against M. Tuberculosis. In three drugs category, the maximum resistance seen against rifampicin, isoniazid & ethambutol and lowest resistance against rifampicin, ethambutol & pyrazinamide.
11. Overall study of trend of resistance prevalence demonstrate the gradually decline in the resistance from November, 2004 to December, 2005. The reduction in resistance against the number of drugs and increase in the sensitivity was fairly observed during aforesaid period. That gave certain lessons to design goals to control resistance in this area. That may also be derived by experiences of successful strategy of rapid implementation of treatment protocol established by DOTS. Moreover; the standardized therapy and continuous follow up is alarming the increase in drug resistan.
12. The ultimate highest level of resistance of rifampicin resistant Mycobacterium Tuberculosis studied by preparing five gradually increasing concentrations. Maximum resistance observed in 1st& 2 nd drug level and minimum at above than 5 th rifampicin level. Maximum sensitivity observed at above 5 th level and zero sensitivity at 1st& 2 nd drug level.

13. In the case of isoniazid, maximum resistance observed in 1st& 2 nd drug level and minimum at above than 5 th level. Maximum sensitivity seen at above 5 th level and zero sensitivity at 1st& 2 nd level.
14. In case of ethambutol, maximum resistances observed in 1st& 2 nd level and minimum at 5 th level. Maximum sensitivity observed at 5 th level and zero sensitivity at 1st& 2 nd level.
15. Maximum resistances observed in 1st& 2 nd pyrazinamide level and minimum at above than 5 th level. Maximum sensitivity seen at above than 5 th pyrazinamide level and zero sensitivity at 1st& 2 nd level.
16. By the overall comparison of resistance of Mycobacterium Tuberculosis against 1stline anti-Tuberculosis drugs, the highest resistance observed at 1st& 2 nd drugs levels and least resistance was founded 3at higher than 5 th drugs level. Most of the resistant Mycobacteriums were inhibited at 3 rd drug levels of their respective anti-Tuberculosis drugs concentrations.
17. First line antituberculisis drugs should therapeutically be interpreted to study pharmacological suitability of dosage and regimen. The pharmacodynamic parameters - Cmax (maximum plasma concentrations), 3therapeutic index and standard dose, maximum regimens, unwanted effects etc considered for accurate scientific outcomes. 13Drug-resistant Tuberculosis poses a significant threat to human health and it is important to understand how the resistance emerges if we are to reverse the upward trend. No any rifampicin resistant train inhibited3at 1stand 2 nd drug levels. 40.54% resistant Mycobacterium -Tuberculosis strains inhibited at 3 rd rifampicin leve. Practically it is not feasible to maintain a plasma concentration higher than therapeutic range of 6.5±3.5ug/ml. 35.12% resistant Mycobacterium Tuberculosis were inhibited at 4 th rifampicin leval., 18.91% inhibited at 5 th rifampicin level and 5.40% inhibited at higher than 5 th rifampicin. These final concentrations incorporated in LJ media exceed the therapeutic index, that's why can not be used in actual practice. The possible unwanted effects are rashes, nausea, vomiting, GI upset, hepatitis, fever and flu like syndrome.
18. No any isoniazid resistant strain inhibited at 1st, 2 nd and 3 rd drug levels. 28% resistant Mycobacterium-Tuberculosis strains inhibited at 4 th isoniazid level. Maximum plasma concentration that can be maintained in body is - 4ug/ml. Therefore it can be used in actual practice. The maximum isoniazid regimen is 400mg. 24% resistant Mycobacterium These concentrations incorporated in LJ media exceed the therapeutic index. Therefore should not be maintained in actual tubercular treatment. The possible unwanted effects are hypersensitivity, paresthenia, hepatic enzyme elevation, hepatitis and peripheral neuropathy, nausea, vomiting, epigastric distress, agranulocytosis, thrombocytopenia, fever, various rashes, dermatological and rheumatoid and lupoid syndromes may produce serious health hazards. The adverse effects are related to dosage and duration of administration. Paresthenia is the most common adverse effect, appears to be due to a relative pyridoxine deficiency.
19. No any ethambutol resistant strain inhibited 3at 1stand 2 nd drug levels 2ug/ml and 4ug/ml. 50% resistant Mycobacterium Tuberculosis strains inhibited at 3 rd level of 6ug/ml. The maximum plasma concentration (Cmax) that can be maintained in Tuberculosis patient during treatment protocol are described by other researchers as 3-5ug/ml (Bertram G. Katzung, 2004), 2-5ug/ml (Leon et al., 2004) and 4-6ug/ml (Richard et al., 2006). It is very obvious from given data, exceeding the mentioned plasma concentration may introduce toxicity. The most important adverse effect of ethambutol is optic neuritis, diminished visual acuity and red-green color blindness. In addition, urate excretion decreased that may be exacerbated the gout. Drug fever, abdominal pain, headache, dizziness and confusion also observed The respective plasma concentration can not be maintained without the risk of sever patient's health hazards. Therefore regimens are rejected to use in actual chemotherapeutical practice.

20. No any pyrazinamide resistant strain inhibited 3at 1stand 2 nd drug levels. 27.66% pyrazinamide resistant Mycobacterium Tuberculosis strains inhibited at 3 rd pyrazinamide level of 300ug/ml. The maximum plasma concentration than can be maintained in human body reported by different researchers are 9-12ug/ml (Joel et al, 2001), 19ug/ml (Leon et al., 2004), 30-50ug/ml (Bertram, 2004), 37-40ug/ml (Richard et al., 2006). Exceeding of this maximally permitted plasma concentration may introduce sever health hazards i.e. nausea, hyperuricemia, rashes, joint ache, gout (rare), fever, anorexia, malaise, hepatitis with or without hepatic jaundice, and death can occur. Maintenance of aforesaid plasma concentration is therefore rejected to use in actual chemotherapeutical practice.

RECOMMENDATIONS

Based upon the findings of the present study, the recommendations can be divided into following major categories:

General Recommendations for Tuberculosis

1. Implementation of standard infection controlling procedures and guidelines to reduced the spread of Tuberculosis pathogenicity in and outside the institutions/ hospitals. ‣ It is highly required to introduce a national mycobacterium infection controlling and molecular resistance surveillance program that is not currently exist in Pakistan. The computer technology and medical informatics may be used for accurate data collection, efficient characterization and timely analysis to successfully overcome the global Tuberculosis burden.
2. The systematic surveillance, quality assurance and digital data support needed for decision making regarding the chemotherapy and/ or prophylaxis purpose of susceptible and resistant Mycobacterium Tuberculosis. It will be forthcoming to predict emerging resistance among available anti-tubercular drugs, leading to effective treatment that could control dissemination of Mycobacterium resistance.
3. Understanding the physiology/ morphology, ecology, genetic and molecular basis of resistance of Mycobacterium Tuberculosis should be increased for successful control of this dangerous microbe.
4. Strengthening the curriculum of human health care professionals in the area of disinfection, house keeping, sterilization and factors contributing to its spread, including inappropriate treatment protocols with ineffective regimens. ‣ Involving the drugs experts (particularly the qualified pharmacists), governmental agencies and welfare organizations to assure the quality medication, therapeutical modifications and implementing the , prophylaxis procedures against Tuberculosis.
5. Establishing the laboratory standards for efficient and accurate detection of molecular and genetic basis of resistance of Mycobacterium Tuberculosis. These standards include sample collection, transportation, physical examination, molecular identification, genetic characterization and reporting techniques used for molecular epidemiology.

Recommendations to improving the awareness against Tuberculosis

6. Educating the patients regarding appropriate use of 1stline drugs against susceptible and 2 nd line treatment protocol against resistant Mycobacterium Tuberculosis.
7. Emphasizing over the research based rapid, reliable diagnostic test and vaccination for prevention and control of mycobacterium infection.
8. Interventioning the health care professionals to reduce the probability to develop resistance to anti-Tuberculosis drugs. That adversely influences the quality of health care outcomes. A well organized and deep rooted intervention can produce surprising benefits.
9. Establishing effective monitoring of drugs used againt Tuberculosis in society.
10. Conducting studies to update the professional knowledge improve the behaviors and inculcate the importance of completion of treatment course to control the tubercular infection in community and hospitals.

11 Increasing the awareness of complications and threats posed by resistant Mycobacterium Tuberculosis in community.

12 Emphasizing to identifying the potential Mycobacterium Tuberculosis strains accurately with molecular

13 Ensuring crucially the understanding and participation in efforts to control the spread of resistance against anti-Tuberculosis agents. Educational programs describe the complications and provide guidelines for using the antitubculosis drugs in general practice and within institutional health care settings.

Recommendations for Prudential Treatment Practice

14 Selecting carefully the treatment protocol and respective dose of anti-Tuberculosis drugs. The medication decision is optimized on basis of pattern of sensitivity of patient's infected Mycobacterium Tuberculosis.

15 A high cure rate without the emergence of resistance can be achieved by adopting the treatments of internationally approved dose/ regimens. Which also are effective in prevention of the resistance emergence. Moreover; the combination chemotherapies minimize the probability of spontaneous mutant to develop resistance to all of the components of chemotherapy.

16 Establishing the base line of effectiveness of anti-Tuberculosis agents, that is compare with: specifications founded in literature, data obtained from therapeutical & resistance surveillance studies and comparative data used to assess the international and/ or local risk factors.

Recommendations related to Epidemiology and resistance prevalence

17 Restricting the over/ under compliance anti-Tuberculosis drugs usage by offering the qualified pharmacist to play their professional role to develop the successful strategies. Pharmacist audit the prescriptions, evaluate the adherence to pharmacological considerations and formulary constrains to stop mechanism of resistance development.

18 Convincing the drugs experts (pharmacists) and clinicians that anti-Tuberculosis drugs therapy should be more closely tailored to the patients requires scientific proof. Prescription practice demands a strong mycobacterium profile, careful laboratory support and confirmed molecular elucidations.

19 Establishing the concurrent morphological, molecular and genetic profile to assess the relationship between Mycobacterium Tuberculosis emerging in treatment premises and community in various environments.

20 Studying systematically to map the prevalence of bacterial pattern of resistance.

21 Standardizing the methods used for diagnosis and sensitivity evaluation with the reference quality tests, which should closely follow the documents published by World Health Organization.

22 Interpreting the correct culture and sensitivity results.

23 Getting immunization against Tuberculosis by BCG vaccination and improving the socio-economic status.

Therapeutical and Mycobacterium resistance management

24 Characterizing and identification of Mycobacterium Tuberculosis strains at primary health care level to coordinate the Tuberculosis patient with the genotyping of detected strain. It will help in anti-tubercular drugs usage, monitoring and intervention programs at institution level.

25 Facilitating to prescribe and/ or decide the treatment protocols with respect of mycobacterial clinical elucidations and therapeutical outcomes of pharmacological studies with the aim of improving the rational selection of regimens.

APPENDICES

Appendix I. Standard Method of Collecting the Sputum Specimen from Tuberculosis Suspected Patient.

Following guidelines strictly implemented to assure quality working,

1. Be cooperative and kind with patient. Improve his confidence, asking him for having the seat, behaving sympathetically.
2. Educate the patient for providing the accurate specimen and importance of pathological evaluations.
3. Use open mouth, unbreakable, screw caped and sterilized sputum container to collect the specimen.
4. Collect the three specimens 1) first spot specimen: when patient come to you first time, 2) early morning specimen and 3) second spot specimen: when patient bring his early morning specimen.
5. Carry the patient in isolation in open air sample collecting place and keep him away from general community to avoid its spreading to other people.
6. Collect the sputum of inner lungs instead of saliva or watery/ foamy exudates.
7. Provide another sputum collecting bottle to patient to bring the early morning sputum in coming day.
8. Guide the patient to wash moth with fresh water, cough to expel forcefully the sputum into the sputum collecting bottle.
9. Ask patient to breathe deeply and then cough, take a cup of ginger and black pepper extract to enhance the chances the maximum sputum expulsion.
10. Always stand at behind of the patient to avoid infection.
11. Collect the sputum containing bottle from patient wash with cloth or cotton place at examining place. Then wash hands and other involve material to assure biosafety.
12. Mark the bottle, write down the specimen’s registration number and enroll the patients in laboratory record.

Appendix II. READING & RECORDING THE SENSITIVITY RESULTS OF TUBERCULOSIS DIAGNOSED PATIENTS

RECORDING THE RESEARCH DATA

"Prevalence, characterization and clinical evaluation of indigenous
Mycobacterium Tuberculosis"

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DRUG SENSITIVITY REPOT:

No.: ______________________________ Date:______________________________
Culture No. : ______________________ Sample: ____________________________
Source: ___________________________ File # : ____________________________

PATIENT PROFILE:

Patient name:____________________________S/D/O______________________________
Indoor: ☐ Out door: ☐ Male: ☐ Female ☐.
Address: ____
Culture date: __________________________ Reading date: _______________________

RESULTS :

Control :10^3 ☐ 10^5 ☐ CS5-10^3 ☐
CS5-10^5 ☐ NB-10^3 ☐ PNB-10^5 ☐

SENSITIVITY/ RESISTANCE:

RFP (R/S):-	G:-
RFP (R/S):-	G:-
RFP (R/S):-	G:
RFP (R/S):-	G:

Date: _________________________________

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RFP- Rifampicin; PZA- Pyrazinamide; BW- Bronchial Wash; PS- Puss; SPT-Sputum; G – Growth; NG- Not growth; Cont- control; Ctm – Contamination; ISN- Isoniazid; CIP-Ciprofloxacin; ETuberculosis- Ethambutol; STP-Streptomycin; PF – Pulmonary fluid

Appendix III. STANDARD OPERATING PROCEDURE (SOP) QUALITY CONTROL MEASURES.

The laboratories staff, workers and executive staff have to adopt all of the possible safely measures and follow the standard operating procedures to avoid the transmission of this mortal pathogens. The major instructions are as given below,

1. Use the mask, overall and gloves to avoid the direct pathogenic contact.
2. Be disinfecting the premises before and after working/ exposing the *Mycobacterium Tuberculosis* in area.
3. Always use the laminar flow hood maintained at negative air pressure and comprised of UV light, gas burner and disinfecting chemicals.
4. Never eat, drink or smoke in laboratory area.
5. Be disinfecting the towel, tools, apparatuses and daily utensils regularly as scheduled in laboratory program.
6. Strictly maintain high quality housekeeping.
7. Be sterilizing the all involved material and apparatuses by using the autoclave.
8. Be assuring the proper, smooth and quality supply of water, gas and other material supply in laboratory.

The resistant M. tuberculosis strains are potential threats for public health. These dangerous superbugs need special attention. The Multi-Drug Resistant and Extensive Drug Resistant M. Tuberculosis are emerging rapidly because of non-compliance and unsupervised indiscriminate use of antibiotics. The cases of tuberculosis cases have been reported in highly susceptible population groups including the local population of Pakistan. Tuberculosis is one of the major public health problems in Pakistan, that ranks 5th amongst high burdened countries of the world. There are 61% of the Tuberculosis patients in the WHO Eastern Mediterranean Region. Approximately 420 000 new tuberculosis cases emerge every year and half of these are sputum smear positive. Pakistan is also estimated to have the 4th highest prevalence of multidrug resistance globally. Hence, an effective strategy is desired to control tuberculosis in Pakistan. Therefore, we have tried to present comprehensive clinical and microbiological information related to M. tuberculosis. That may helps to develop the required skills to achieve the Millennium Development Goals of World Health Organization.

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